REMARKS/ARGUMENTS

Claims 48-59 are pending in the present application.

OBVIOUSNESS-TYPE DOUBLE PATENTING REJECTION OF THE CLAIMS

Claims 48-55 and 57-59 are rejected under the judicially created doctrine of obviousness type double patenting rejection over claims 1-3, 5-7 and 15 of application 09/757,788 ("the '788 application"). In particular, the Examiner alleges that "because the instant application discloses the same formulation containing the same GLP-1 compound as the application 09/757,788 and the specification of the instant applications also indicates that the GLP-1 compound can be used for nasal administration, thus it would be obvious that the formulation would achieve a mass median aerodynamic diameter of less than 10 um upon nebulization" (page 4 of Office Action).

Applicants respectfully traverse this rejection and submit that the Examiner has failed to present a prima facie case for why a person of ordinary skill in the art would conclude that the invention defined in claims 48-55 and 57-59 of the present application would be an obvious variation of the invention defined in claims 1-3, 5-7 and 15 of the cited '788 application.

In addressing the above obviousness-type double patent rejection, Applicants present below a side by side comparison of the limitations of independent claim 48 of the present application with the limitations of independent claim 1 of the '788 application:

Claim 48 of the present application	Claim 1 of the 788 application
1) A **GLP-1 derivative**	1) A **liquid formulation** suitable for pulmonary administration comprising a GLP-1 compound
2) having a lipophilic substituent of **8-40 carbon atoms**	2) having a lipophilic substituent of **14-18 carbon atoms**

3) attached to the **C-terminal AA** of GLP-1	3) attached to **any AA** of GLP-1
4) optionally via a spacer	4) optionally via a spacer
	5) **wherein said formulation upon nebulization achieves a mass median aerodynamic diameter of less than 10 um.**

Thus, looking at the above comparison of limitations between the claims of the pending application and the claims of the '788 application, the question to be addressed is whether **claims** 48-55 and 57-59 of the present application are rendered obvious by **claims** 1-3, 5-7 and 15 of the 788 application. Applicants submit, for the reasons set forth below, that the clear answer to this question must be in the negative.

The Examiner alleges that "the instant application discloses the same formulation containing the same GLP-1 compound as the application 09/757,788" (page 4 of Office Action). However, reference to the above comparison between the claims of the present application and the claims of the '788 application reveals that "the same formulation containing the same GLP-1 compound" is clearly not claimed in the two applications.

First, the claims in the present application are to GLP-1 derivatives and **not** to formulations of any kind. By comparison, the claims in the '788 application are to liquid formulations suitable for pulmonary administration where such formulations contain GLP-1 compounds. **Thus, the claims in both applications are not directed to formulations.**

Second, the Examiner has provided no reasoning for why the GLP-1 compound contained in the liquid formulations claimed in the 788 application (i.e., a GLP-1 compound which has a lipophilic substituent of **14-18 carbon atoms attached to any AA** of the GLP-1 compound) would render obvious a GLP-1 derivative in which a lipophilic substituent **of 8-40 carbon atoms is attached to the C-terminal AA** of the GLP-1 or analog thereof (i.e., the claims of the present application).

Instead, the Examiner opines that the claims of the present application are obvious over the claims of the '788 application because the present application discloses that the GLP-1 derivatives described therein can be used for nasal administration and "thus it would be obvious that the formulation would achieve a mass median aerodynamic diameter of less

than 10um upon nebulization" (page 4 of Office Action). This argument is wrong both legally and factually.

First, the issue to be addressed here is whether a liquid formulation that contains GLP-1 compounds and which achieves a mass median aerodynamic diameter of less than 10um upon nebulization (i.e., the claims of the 788 application) would render obvious the GLP-1 derivatives claimed in the present application **and not, as suggested by the above quote from the Examiner,** whether a nasal formulation of the GLP-1 derivatives claimed in the present application would achieve mass median aerodynamic diameter of less than 10um upon nebulization[1] (the MMAD requirement being a limitation of the '788 claims and not of the claims pending in this application).

Second, while the specification of a patent or application cited as the basis for an obviousness-type double patenting rejection (here, the '788 application) may be relied on in certain limited circumstances (see MPEP 804), the nasal formulation disclosure relied on by the Examiner is from the specification of the present application and not from the cited '788 application and thus its citation is improper as a matter of law and PTO policy.

Third, as noted above, the claims of the present application are to GLP-1 derivatives and not to formulations. Thus, the issue to be addressed is whether the claims of the '788 application render obvious the GLP-1 derivatives claimed in the present application; not whether the claims of the 788 application render obvious a nasal formulation of the GLP-1 derivatives claimed in the present application.

In sum, the Examiner's reliance on the disclosure in the specification of the present application of nasal formulations is improper and misplaced and does not provide any reason for why a person of ordinary skill in the art would conclude that the invention defined in claims 48-55 and 57-59 of the present application would be an obvious variation of the invention defined in claims 1-3, 5-7 and 15 of the cited '788 application. Accordingly, in view of the above remarks, Applicants submit that the claims of the present application are not obvious over the claims in application 09/757,788 and withdrawal of this rejection is

1 In any event, Applicants note that while the liquid formulation suitable for pulmonary use in the '788 claims is required to achieve an MMAD of less than 10 um upon nebulization, an MMAD of greater than 10-20 um is recommended for nasal administration (see, for example, the first paragraph on page 11 of the attached 1999 draft Guidance For Industry from the FDA which in turn cites to the 1966 Task Group On Lung Dynamics)

therefore respectfully requested.

REJECTION OF THE CLAIMS UNDER 35 U.S.C. 102

Claims 48-49 are rejected under section 102 (b) over Habener US Patent 5,118,666 ("the '666 patent") based on the Examiner's assertion that the reference in Habener to "lower alkyl" would be understood in the art (citing to US patent 3,567,450 entitled "Photoconductive Elements Containing Triarylamine Photoconductors") to be C_1-C_8 or C_1-C_{12}. In particular, the Examiner states that the phrase "alkyl can be C_1-C_{12}" (col. 6, lines 25-26) in Habener merely identifies the alkyl group being C_1-C_{12} in the compounds of tetraalkylammonium and trialkylammonium and therefore, that "the term is not relevant to whether the C_1-C_{12} is lower alkyl or not" (page 5 of Office Action). The Examiner also dismisses Applicants citation to US patent 6,828,303 as evidence that "lower alkyl" in the Habener '666 patent means C_1-C_6 alkyl because the '303 patent is a post filing reference.

Applicants respectfully traverse this rejection.

First, Applicants cited to the phrase "alkyl can be C_1-C_{12}" (col. 6, lines 25-26) in the Habener '666 patent as evidence that the use of "lower alkyl" elsewhere in the '666 patent must mean something less than 12 carbons. It is Applicants' position that to suggest, as the Examiner has done, that the phrases "alkyl" and "lower alkyl" in the same patent could each mean the same thing (i.e. a C_1-C_{12} group) would deprive the word "lower" when used in conjunction with "alkyl" of any meaning.

Second, the Examiner's dismissal of US patent 6,828,303 as evidence that "lower alkyl" in the Habener '666 patent means C_1-C_6 alkyl because the '303 patent is a post filing reference is obviated by the fact that the European counterpart of the '303 patent (EP 619322, copy attached) published in October 1994; i.e., prior to the March 17, 1995 priority filing date of the present application. Thus, the disclosure on page 2, lines 29-37 of EP 619322 that the "lower alkyl" group of the '666 patent is a C_1-C_6 alkyl **provides clear evidence that**

prior to the filing date of the present application, "lower alkyl" in the Habener '666 patent was understood to mean C_1-C_6 alkyl by those skilled in the art in the field of GLP-1 peptides.

Third, with respect to the Examiner's assertion that US patent 3,567,450 provides evidence that the art recognizes that "lower alkyl" can be C_1-C_8 or C_1-C_{12}, the 450 patent only discloses that lower alkyl is C_1-C_8 (see col. 3, lines 26-28 and claim 1) and moreover, the '450 patent (like the '458 patent cited by the Examiner in the previous Office Action in support of the same assertion) is not relevant to what one skilled in the art in the field of GLP-1 peptides would understand the phrase "lower alkyl" in the Habener '666 patent to mean as the '450 patent is directed to photoconductive elements for use in photoconductors. In regard to this latter point, Applicants note that the Examiner did not contest Applicants' characterization in the previous Amendment of the previously cited '458 patent as irrelevant to what one skilled in the art in the field of GLP-1 peptides would understand the phrase "lower alkyl" to mean (the'458 patent being directed to emulsified spray formulations of herbicides or pesticides) but instead cited a patent (the '450 patent) that is even less relevant to the field of GLP-1 peptides in the present Office Action.

In sum, it is Applicants' position that the Examiner's reference to the '450 patent (entitled "Photoconductive Elements Containing Triarylamine Photoconductors") as evidence that "lower alkyl" in the Habener '666 patent meant C_1-C_8 or C_1-C_{12} cannot be relied on to maintain the rejection of claims 48-49 as anticipated by Habener in light of the above-cited disclosure in EP 619322 which **provides clear evidence that prior to the filing date of the present application, "lower alkyl" in the Habener '666 patent was understood to mean C_1-C_6 alkyl by those skilled in the art in the field of GLP-1 peptides.**

Accordingly, Applicants respectfully request withdrawal of the rejection of claims 48-49 of the present application as anticipated by the Habener '666 patent.

In sum, in view of the above remarks, Applicants respectfully submit that this application is in condition for allowance and early favorable action to that effect is solicited.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Please charge any deficiencies or overpayment to Deposit Account No.14-1447.

Respectfully submitted,

Date: December 21, 2006

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(54) **Prolonged delivery of insulinotropin (GLP-1)**

Verzögerte Freigabe von Insulinotropin (GLP-1)

Libération prolongée d'insulinotropine (GLP-1)

(84) Designated Contracting States:
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(56) References cited:
WO-A-90/11296
WO-A-91/11457
WO-A-93/18785
US-A- 5 118 666

- **METABOLISM, vol. 42, no. 1, January 1993 pages 1-6, G.K. HENDRICK ET AL. 'Glucagon-like Peptide I (7-37) suppresses hyperglycemia in rats'**

EP 0 619 322 B1

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Description

[0001] The present invention relates to compositions useful for the treatment of Diabetes Mellitus. More specifically, the present invention relates to compositions to prolong the administration of glucagon-like peptide 1 (GLP-1), and derivatives thereof. These compositions are useful in treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM).

[0002] The amino acid sequence of GLP-1 is known as:

His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 1)

[0003] GLP-1 is disclosed by Lopez, L.C., et al., P.N.A.S., USA 80: 5485-5489 (1983); Bell, G.I., et al., Nature 302: 716-718 (1983); Heinrich, G. et al., Endocrinol. 115: 2176-2181 (1984) and Ghiglione, M., et al., Diabetologia 27: 599-600 (1984).

[0004] During processing in the pancreas and intestine, GLP-1 is converted to a 31 amino acid peptide having amino acids 7-37 of GLP-1, hereinafter this peptide is referred to as GLP-1 (7-37).

[0005] This peptide has been shown to have insulinotropic activity, that is, it is able to stimulate, or cause to be stimulated, the synthesis or expression of the hormone insulin. Because of this insulinotropic activity, GLP-1 (7-37) is alternatively referred to as insulinotropin.

[0006] GLP-1 (7-37) has the following amino acid sequence:

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2).

[0007] GLP-1 (7-37), certain derivatives thereof and the use thereof to treat Diabetes mellitus in a mammal are disclosed in United States Patent Numbers 5,118,666 ('666 patent) and 5,120,712 (712 patent).

[0008] The derivatives of GLP-1 (7-37) disclosed in the '666 and '712 patents include polypeptides which contain or lack one of more amino acids that may not be present in the naturally occurring sequence. Further derivatives of GLP-1 (7-37) disclosed in the '666 and '712 patents include certain C-terminal salts; esters and amides where the salts and esters are defined as OM where M is a pharmaceutically acceptable cation or a lower (C_1-C_6) branched or unbranched alkyl group and the amides are defined as $-NR^2R^3$ where R^2 and R^3 are the same or different and are selected from the group consisting of hydrogen and a lower (C_1-C_6) branched or unbranched alkyl group.

[0009] Certain other polypeptides, alternatively referred to as truncated GLP-1 or truncated insulinotropin, having insulinotropic activity and the derivatives thereof are disclosed in PCT/US 89/01121 (WO 90/11296). Those polypeptides, referred to therein as GLP-1 (7-36), GLP-1 (7-35) and GLP-1 (7-34) have the following amino acid sequences, respectively.

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3);

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly (SEQUENCE ID NO: 4);

and

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

[0010] Derivatives of the polypeptides disdosed in WO 90/11296 include polypeptides having inconsequential amino

acid substitutions, or additional amino acids to enhance coupling to carrier protein or to enhance the insulinotropic effect thereof. Further derivatives of insulinotropin disclosed in WO 90/11296 include certain C-terminal salts, esters and amides where the salts and esters are defined as OM where M is a pharmaceutically acceptable cation or a lower branched or unbranched alkyl group and the amides are defined as $-NR^2R^3$ where R^2 and R^3 are the same or different and are selected from the group consisting of hydrogen and a lower branched or unbranched alkyl group.

[0011] WO 91/11457 and WO 93/18785 disclose sustained release compositions of anti-diabetic GLP-1 peptides.

[0012] In the following, reference is made to the accompanying drawings, in which:

Fig. 1 shows the effect of a prolonged infusion (7 hours) of 4 ng/kg/min insulinotropin on plasma glucose levels in patients with NIDDM.
Fig. 2 shows the effect of a short infusion (60 minutes) of 10 ng/kg/min insulinotropin on plasma glucose levels in patients with NIDDM.
Fig. 3 shows the effect of a prolonged infusion (7 hours) of 2 ng/kg/min and 4 ng/kg/min of insulinotropin on plasma glucose levels in patients with NIDDM.
Fig. 4. Mean (n=3) Plasma Concentration of Insulinotropin in Rats After Subcutaneous Administration of Single 0.5 mg/0.5 ml Doses in Different Aqueous Suspensions (AS).
Fig. 5. Mean (n=3) Plasma Concentration of Insulinotropin in Rats After Subcutaneous Administration of Single 0.5 mg/0.5 ml Doses in Different Aqueous Suspensions (AS).
Fig. 6. Mean (n=3) Plasma Concentration of Insulinotropin in Rats After Subcutaneous Administration of Single 0.5 mg/0.5 ml Doses in Different Aqueous Suspensions (AS).
Fig. 7. Mean (n=3) Plasma Concentration of Insulinotropin in Rats After Subcutaneous Administration of Single 0.5 mg/0.5 ml Doses in Different Aqueous Suspensions (AS).
Fig. 8. Mean (n=3) Plasma Concentration of Insulinotropin in Rats After Subcutaneous Administration of Single 0.5 mg/0.13 ml Doses in Different Aqueous Suspensions (AS).
Fig. 9. Mean (n=3) Plasma Concentration of Insulinotropin in Rats After Subcutaneous Administration of Single 0.5 mg/0.13 ml Doses in Different Aqueous Suspensions (AS).
Fig. 10 shows pharmacokinetic studies of an insulinotropin zinc precipitate.

In a first aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;

(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a polymer selected from polyethylene glycol, polyoxyethylene-polyoxypropylene copolymers, polyanhydrides and polysaccharides, wherein said polysaccharides are selected from chitosan, acacia gum, karaya gum, guar gum, xanthan gum, tragacanth, alginic acid, carrageenan, agarose, and furcellarans, dextran, starch, starch derivatives and hyaluronic acid;

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and has been treated in such a way that it comprises said compound of part (i) in crystalline or amorphous form having a solubility equal to or less than 500 µg/ml under physiological conditions.

In a second aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a pharmaceutically acceptable water-immiscible oil suspension; said oil selected peanut oil, sesame oil, almond oil, caster oil, camellia oil, cotton seed oil, olive oil, corn oil, soy oil, safflower oil, esters of fatty acids, and esters of fatty alcohols

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in particulate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

The composition of the second aspect may further comprise (i) a wetting agent which is a non-ionic surfactant and (i) a suspending agent.

In a third aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;

(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from :

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or and C_1-C_6 dialkyl amide of said peptides, and

(ii) zinc (II), which is mixed with the peptide;

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in crystalline or amorphous form having a solubility equal to or less than 500 µg/ml under physiological conditions.
The zinc may be amorphous or crystalline.
In a fourth aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from :

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a metal selected from Ni(II), Co(II), Mn(II), Fe(II), and Cu(II),

wherein said composition of matter is in an injectable formulation and comprises said compound of part (i) in crystalline or amorphous form having a solubility equal to or less than 500 µg/ml under physiological conditions.

In a fifth aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE NO: 2, SEQUENCE NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) phenol, cresol, resorcinol or methyl paraben,

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

In a sixth aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;

wherein X is :

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a basic polypeptide and a phenolic compound,

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

In a seventh aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a basic polypeptide, a phenolic compound, and a metal ion;

wherein said composition of matter is in injectable form, is capable of achieving sustained glycaemic control and comprises said compound of part (i) precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.
The basic polypeptide may be protamine, and the metal ion may be zinc.
In an eighth aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides ,

said peptides and derivatives thereof having being subjected to conditions resulting in amorphous or crystalline precipitates or aggregates having a solubility equal to or less than 500 µg/ml under physiological conditions; wherein said conditions are high shear, exposure to salts; or combinations thereof;

wherein said composition of matter is in an injectable formulation and is capable of achieving sustained glycaemic control.
In the composition of the eighth aspect, the salt may be is ammonium sulphate, sodium sulphate, lithium sulphate, lithium chloride, sodium citrate, ammonium citrate, sodium phosphate, potassium phosphate, sodium chloride, potassium chloride, ammonium chloride, sodium acetate, ammonium acetate, magnesium sulphate, calcium chloride, ammonium nitrate, sodium formate, or a combination thereof.
In a ninth aspect, the present invention provides a composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is :

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a basic polypeptide;

wherein said composition of matter is in injectable form, is capable of achieving sustained glycaemic control and comprises said compound of part (i) precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.
The basic polypeptide may be protamine.
In a further aspect, the invention provides a composition of the first to ninth aspects for use in medicine. In a yet further aspect, the invention provides the use of a composition of the first to ninth aspects in the manufacture of a medicament for treating non-insulin dependent diabetes mellitus.
The medicament may be for administration which is subcutaneous, intramuscular, transdermal, by an infusion pump, by oral inhalation, by nasal inhalation, or gastrointestinal.
The prolonged administration of the compositions of the present invention can be used for the treatment of non-insulin dependent diabetes mellitus in a mammal.

[0013] Unless otherwise indicated, the term "derivative", as used throughout this Specification and the appendant claims, includes, but is not limited to, polypeptides comprising the primary structure shown, wherein one or more L-amino acids are included at the C-terminus thereof; wherein the C-terminal carboxyl group forms an ester with a (C_1-C_6) straight or branched chain alkyl group; wherein the C-terminal carboxyl group forms a carboxamide or substituted carboxamide; wherein the acidic amino acid residues (Asp and/or Glu) form an ester or carboxamide; and combinations thereof.

[0014] Included within the scope of this invention are polypeptides having homology to the peptides described above, which homology is sufficient to impart insulinotropic activity to such polypeptides. Also included within the scope of this invention are variants of the polypeptides described above, which variants comprise inconsequential amino acid substitutions and have insulinotropic activity.

[0015] Glucagon-like Peptide-1 (7-37), its isolation, characterization, and use to treat Diabetes mellitus are disclosed in United States Patent Number 5,118,666 and 5,120,712.

[0016] In the present invention, it has now been discovered that prolonged plasma elevations of GLP-1, and related polypeptides, are necessary during the meal and beyond to achieve sustained glycemic control in patients with Non Insulin Dependent Diabetes Mellitus. It has surprisingly been found that raising GLP-1, and related peptides, around meal time alone, even for periods of up to one hour, will not adequately control the glucose levels. Thus, administration

of GLP-1, and related peptides, requires a prolonged delivery system. This prolonged delivery system leads to an enhancing of insulin action.

[0017] The phrase "enhancing insulin action", as used throughout this Specification and the appendant claims, includes, but is not limited to, one or more of increasing insulin synthesis, increasing insulin secretion, increasing glucose uptake by muscle and fat and decreasing glucose production by the liver.

[0018] The polypeptides of this invention are prepared by various methods well known to those skilled in the art. For example, the polypeptides can be synthesized using automated peptide synthesizers such as an Applied Biosystems (ABI) 430A solid phase peptide synthesizer. Alternatively, the polypeptides of this invention can be prepared using recombinant DNA technology wherein a DNA sequence coding for the polypeptide is operably linked to an expression vector and used to transform an appropriate host cell. The transformed host cell is then cultured under conditions whereby the polypeptide will be expressed. The polypeptide is then recovered from the culture. Further still, a combination of synthesis and recombinant DNA techniques can be employed to produce the amide and ester derivatives of this invention and/or to produce fragments of the desired polypeptide which are then joined by methods well known to those skilled in the art.

[0019] Derivatives of the polypeptides according to this invention are prepared by methods well known to those skilled in the art. For example, C-terminal alkyl ester derivatives of the polypeptides of this invention are prepared by reacting the desired (C_1-C_6)alkanol with the desired polypeptide in the presence of a catalytic acid such as HCl. Appropriate reaction conditions for such alkyl ester formation include a reaction temperature of about 50°C and reaction times of about 1 hour to about 3 hours. Similarly, derivatives of the polypeptides of this invention comprising (C_1-C_6) alkyl esters of the Asp and/or Glu residues within the polypeptide can be so formed.

[0020] Carboxamide derivatives of the polypeptides of this invention are also prepared by solid phase peptide synthesis methods well known to those skilled in the art. For example, see, Solid Phase Peptide Synthesis, Stewart, J.M. et al., Pierce Chem. Co. Press, 1984.

[0021] Alternatively, or in combination with the above, derivatives of the polypeptides of this invention can be prepared by modifying the DNA coding sequence for such polypeptide so that a basic amino acid residue is replaced with a different basic amino acid residue or with an acid acidic or neutral amino acid residue, or an acidic amino acid residue is replaced with a different acidic amino acid residue or with a basic or neutral amino acid residue, or a neutral amino acid residue is replaced with a different neutral amino acid residue or with an acidic or basic amino acid residue. Such changes in polypeptide primary sequence can also be accomplished by direct synthesis of the derivative. Such methods are well known to those skilled in the art. Of course, such derivatives, to be useful in the practice of this invention, must achieve an insulinotropic effect.

[0022] The insulinotropic activity of a polypeptide derivative according to this invention is determined as follows.

[0023] Pancreatic islets are isolated from pancreatic tissue from normal rats by a modification of the method of Lacy, P.E., et al., Diabetes, 16:35-39 (1967) in which the collagenase digest of pancreatic tissue is separated on a Ficoll gradient (27%, 23%, 20.5% and 11% in Hanks' balanced salt solution, pH 7.4). The islets are collected from the 20.5%/11% interface, washed and handpicked free of exocrine and other tissue under a stereomicroscope. The islets are incubated overnight in RPMI 1640 medium supplemented with 10% fetal bovine serum and containing 11 mM glucose at 37°C and 95% air/5% CO_2. The islets are then transferred to RPMI 1640 medium supplemented with 10% fetal bovine serum and containing 5.6 mM glucose. The islets are incubated for 60 minutes at 37°C, 95% air/5% CO_2. The polypeptide derivative to be studied is prepared at 1 nM and 10nM concentrations in RPMI medium containing 10% fetal bovine serum and 16.7 mM glucose. About 8 to 10 isolated islets are then transferred by pipette to a total volume of 250 µl of the polypeptide derivative containing medium in 96 well microtiter dishes. The islets are incubated in the presence of the polypeptide derivative at 37°C, 95% air/5% CO_2 for 90 minutes. Then, aliquots of islet-free medium are collected and 100 µl thereof are assayed for the amount of insulin present by radioimmunoassay using an Equate Insulin RIA Kit (Binax, Inc., Portland, ME).

[0024] Dosages effective in treatment of adult onset diabetes will range from about 1 pg/kg to 1,000 µg/kg per day when a polypeptide derivative of this invention is administered, for example, intravenously, intramuscularly or subcutaneously. A preferred dosage range for intravenous infusion during and between meals is about 4 to 10 ng/kg/min or about 0.6 to 1.4 µg/day based on a 100 kg patient. It is to be appreciated, however, that dosages outside of that range are possible and are also within the scope of this invention. The appropriate dosage can and will be determined by the prescribing physician and will be a result of the severity of the condition being treated as well as the response achieved with the derivative being administered and the age, weight, sex and medical history of the patient.

[0025] The prolonged administration may be achieved by subcutaneous, intramuscular, or transdermal means, oral inhalation, nasal inhalation, gastrointestinal, or by means of an infusion pump.

[0026] Prolonged administration of GLP-1, and related peptides, may also be achieved by formulation as a solution in various water-soluble polymers. These polymers are generally low molecular weight (< 15 kDa) polymers. Non-limiting examples of such low molecular weight polymers include polyethylene glycol, polyvinylpyrrolidone, polyvinylalcohol and polyoxyethylene-polyoxypropylene copolymers. Higher molecular weight polymers may be used. Non-

limiting examples of higher molecular weight polymers include polysaccharides such as cellulose and its derivatives, chitosan, acacia gum, karaya gum, guar gum, xanthan gum, tragacanth, alginic acid, carrageenan, agarose, furcelleran. In the later case, polymers which are degraded in vivo either enzymatically or by hydrolysis are preferred, for example, dextran; starch and its derivatives, hyaluronic acid, polyesters, polyamides, polyanhydrides and polyortho esters. The tissue accumulation associated with high molecular weight, non-biodegradable polymers is avoided by using low molecular weight polymers or biodegradable polymers. The formulations typically contain GLP-1, or related peptides, at approximately 1 mg/ml, with concentration dependent on the polymer, but typically at concentrations up to that which will attain a 50 cps viscosity, and possibly a suitable buffer, tonicity agent, and preservative. In vivo data in rats and man demonstrate that the formulations are capable of achieving measurable blood insulinotropin, for example, levels for up to 24 hours. In contrast, insulinotropin, for example, formulated in phosphate-buffered saline results in rapid (~ 15 minutes) peak plasma levels, with plasma level dropping below detection limits in just over 4 hours. Plasma concentration versus time plots suggest that insulinotropin absorption rate, for example, from the injection site has been significantly reduced in the presence of the polymers.

[0027] GLP-1, and related peptides, may also be formulated as particles suspended in a pharmaceutically acceptable oil. The preferred oils are triglycerides. Non-limiting examples of such oils include peanut oil, sesame oil, almond oil, castor oil, camellia oil, cotton seed oil, olive oil, corn oil, soy oil, safflower oil, and coconut oil. Oils of other classes are acceptable, for example, esters of fatty acids and esters of fatty alcohols, as long as the oil is immiscible with water and is a poor solvent for the peptide. The formulation may also contain appropriate preservatives, wetting agents, and suspending agents. The weight percent of insulinotropin, for example, in the formulation may vary from 0.01 to 10%. In vivo data in rats demonstrate that these formulations are capable of achieving measurable insulinotropin blood levels, for example, for up to 24 hours. In contrast, insulinotropin, for example, formulated in phosphate-buffered saline results in rapid (~ 15 minutes) peak plasma levels, with plasma level dropping below detection limits in just over 4 hours. Plasma concentration versus time plots suggest that insulinotropin absorption rate from the injection site have been significantly reduced in the oil suspensions.

[0028] GLP-1, and related peptides, may also be formulated as a low solubility form for administration by combination with a metal ion, preferably in the form of a salt. A preferred ion is zinc (II). The combination may result in a composition which is amorphous or crystalline. Other metal ions may also be used including Ni(II), Co(II), Mg(II), Ca(II), K(I), Mn (II), Fe(II) and Cu(II).

[0029] Another type of prolonged delivery formulation is an aqueous suspension of insulinotropin precipitates or aggregates formed by using precipitants for example, phenolic compounds or basic polypeptides or metal ions or salts, and/or by using high shear. More than one precipitant can be used at one time. The precipitates can be either crystalline or amorphous.

[0030] Insulinotropin crystals can be obtained from a solution of the drug in water by using pH gradient (either high to low or low to high) and/or temperature gradient and/or salts to reduce solubility. The salts include ammonium citrate, sodium or potassium phosphate, sodium or potassium or ammonium chloride, sodium or ammonium acetate, magnesium sulfate, calcium chloride, ammonium nitrate, sodium formate, and any other salts which can reduce the solubility of the drug. If the salt used for crystallization is not pharmaceutically acceptable, the mother liquor can be substituted by pharmaceutically acceptable medium after crystallization is completed. If further reduction of drug solubility is necessary to achieve a desirable pharmacokinetic profile, the crystals can be treated by metal ions such as zinc or calcium and/or phenolic compounds. The treatment can be done by simply incorporating those additives to the crystal suspension.

[0031] The solubility of the insulinotropin precipitates or aggregates can range from less than 1 µg/mL to 500 µg/mL under physiological conditions. In vivo data in rats demonstrate that the formulations are capable of achieving measurable insulinotropin blood levels, for example, for at least 30 hours.

[0032] Aqueous media used for the above formulations can be any kind of buffer system which can be used for injection or even with pure water. The pH of the final formulation can be any value as long as the formulation is injectable. Protamine can be added as any kind of salt form (e.g. sulfate, chloride, etc.) or protamine base. Exemplary concentration ranges of the components which can be used for the formulation preparation are as follows: phenol (0.5 to 5.0 mg/ml), m-cresol (0.5 to 5.5 mg/ml), protamine (0.02 to 1.0 mg/ml), zinc (0.10 to 6 zinc/insulinotropin molar ratio), sodium chloride (up to 100 mg/ml), and phosphate buffer (5-500 mM).

[0033] Other phenolic on non phenolic compounds may also be used. Non-limiting examples of such compounds include resorcinol, methylparaben, propylparaben, benzyl alcohol, chlorocresol, cresol, benzaldehyde, catecol, pyrogallol, hydroquinone, n-propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene. Non-limiting examples of basic polypeptides are polylysine, polyarginine, etc.

[0034] Having described the invention in general terms, reference is now made to specific examples. It is to be understood that these examples are not meant to limit the present invention, the scope of which is determined by the appended claims.

EXAMPLE 1

Insulinotropin (1 mg/ml) Suspension

Solution A1 preparation

[0035] 10 mg of insulinotropin was weighed into a 5 ml volumetric flask. Approximately 4 ml of phosphate buffered saline (PBS) was added to the flask to disperse and dissolve the drug. Sufficient PBS (q.s. amount) was added to fill the flask. 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. The volumes in both flasks were combined by filtering them by a glass syringe through a 0.22 μ filter (low protein binding) into a 10 ml glass vial. Solution A1 contained insulinotropin 2 mg/ml in PBS.

Solution B1 preparation

[0036] 8 mg of protamine sulfate and 44 mg of phenol were weighed into a 10 ml volumetric flask. The q.s. amount of PBS was added to dissolve the protamine sulfate and the phenol. This solution was filtered through a 0.22 μ filter (low protein binding) into a 10 ml glass vial. Solution B1 contained protamine base 0.6 mg/ml and phenol 4.4 mg/ml in PBS.

Aqueous Suspension 1

[0037] 1.5 ml of solution A1 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B1 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred gently for 16 hours to allow suspension formation. Aqueous Suspension 1 contained insulinotropin 1 mg/ml, protamine base 0.3 mg/ml and phenol 2.2 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 2

Insulinotropin (1 mg/ml) Suspension

Solution A2 preparation

[0038] 10 mg of insulinotropin was weighed into a 5 ml volumetric flask. Approximately 4 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of the PBS was added to the flask. The volumes in both flasks were combined by filtering them by a glass syringe through a 0.22μ filter into a 10 ml glass vial. Solution A2 contained insulinotropin 2 mg/ml in PBS.

Solution B2 Preparation

[0039] 2 mg of protamine sulfate and 44 mg of phenol were weighed into a 10 ml volumetric flask. The q.s. amount of PBS was added to the flask to dissolve the protamine sulfate and phenol. This solution was filtered through a 0.22μ filter into a 10 ml glass vial. Solution B2 contained protamine base 0.15 mg/ml and phenol 4.4 mg/ml in PBS.

Aqueous Suspension 2

[0040] 1.5 ml of solution A2 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B2 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 2 contained insulinotropin 1 mg/ml, protamine base 0.075 mg/ml, and phenol 2.2 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 3

Insulinotropin (1 mg/ml) Suspension

Solution A3 preparation

[0041] 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A3 was filtered by a syringe through a 0.22 µ filter into a 10 ml glass vial. Solution A3 contained insulinotropin 2 mg/ml in PBS.

Solution B3 preparation

[0042] 8 mg of protamine sulfate, 44 mg of phenol, and 323 mg of glycerin were weighed into a 10 ml volumetric flask. The q.s. amount of PBS was added to the flask to dissolve the protamine sulfate, the phenol, and the glycerin. This solution was filtered by a syringe through a 0.22 µ filter into a 10 ml glass vial. Solution B3 contained protamine base 0.6 mg/ml, phenol 4.4 mg/ml, and glycerin 32 mg/ml in PBS.

Aqueous Suspension 3

[0043] 1.5 ml of Solution A3 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of Solution B3 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 3 contained insulinotropin 1 mg/ml, protamine base 0.3 mg/ml, phenol 2.2 mg/ml, and glycerin 16 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 4

Insulinotropin (1 mg/ml) Suspension

Solution A4 preparation

[0044] 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A4 was filtered by a syringe through a 0.22 µ filter (Millipore Millex-GV) into a 10 ml glass vial. Solution A4 contained insulinotropin 2 mg/ml in PBS.

Solution B4 preparation

[0045] 8 mg of protamine sulfate and 52 mg of m-cresol were weighed into a 10 ml volumetric flask. The q.s. amount of PBS was added to the flask to dissolve the protamine sulfate and the m-cresol. This solution was filtered through a 0.22 µ filter into a 10 ml glass vial. Solution B4 contained protamine base 0.6 mg/ml and m-cresol 5 mg/ml in PBS.

Aqueous Suspension 4

[0046] 1.5 ml of solution A4 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B4 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow crystal formation. Aqueous Suspension 4 contained insulinotropin 1 mg/ml, protamine base 0.3 mg/ml and m-cresol 2.5 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 5

Insulinotropin (1 mg/ml) Suspension

Solution A5 preparation

[0047] 50 mg of insulinotropin was weighed into a 25 ml volumetric flask. Approximately 23 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A5 was filtered

by a syringe through a 0.22.μ filter into a 50 ml glass vial. Solution A5 contained insulinotropin 2 mg/ml in PBS.

Phenol Stock Solution Preparation

[0048] 0.44 g of phenol was weighed into a 100 ml volumetric flask. Approximately 95 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask to dissolve the phenol. The resulting solution (4.4 mg/ml phenol) was used to prepare Solution B5.

Solution B5 preparation

[0049] Solution B5 was prepared by filtering 25 ml of the phenol stock solution through a 0.2 μ filter into a 50 ml glass vial. Solution B5 contained phenol 4.4 mg/ml in PBS.

Aqueous Suspension 5

[0050] 1.25 ml of solution A5 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.25 ml of solution B5 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 5 contained insulinotropin 1 mg/ml and phenol 2.2 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 6

Insulinotropin (1 mg/ml) Suspension

Solution A6 preparation

[0051] 50 mg of insulinotropin was weighed into a 25 ml volumetric flask. Approximately 23 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A6 was filtered by a syringe through a 0.22 μ filter into a 50 ml glass vial. Solution A6 contained insulinotropin 2 mg/ml in PBS.

Phenol Stock Solution Preparation

[0052] 0.44 g of phenol was weighed into a 100 ml volumetric flask. Approximately 95 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask to dissolve the phenol. The resulting solution (4.4 mg/ml phenol) was used to prepare Solution B6.

Solution B6 preparation

[0053] Solution B6 was prepared by weighing 1.25 mg of protamine sulfate into a 25 ml volumetric flask. Approximately 20 ml of phenol stock solution was added to the flask to dissolve the protamine sulfate. The q.s. amount of phenol stock solution was added to the flask. Solution B6 was filtered through a 0.22 μ filter into a 50 ml glass vial. Solution B6 contained phenol 4.4 mg/ml and protamine base 0.038 mg/ml in PBS.

Aqueous Suspension 6

[0054] 1.25 ml of solution A6 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.25 ml of solution B6 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 6 contained insulinotropin 1 mg/ml, phenol 2.2 mg/ml, and protamine base 0.019 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 7

Insulinotropin (1 mg/ml) Suspension

Solution A7 preparation

[0055] 50 mg of insulinotropin was weighed into a 25 ml volumetric flask. Approximately 23 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A7 was filtered by a syringe through a 0.22 µ filter into a 50 ml glass vial. Solution A7 contained insulinotropin 2 mg/ml in PBS.

Phenol Stock Solution Preparation

[0056] 0.44 g of phenol was weighed into a 100 ml volumetric flask. Approximately 95 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask to dissolve the phenol. The resulting solution (4.4 mg/ml phenol) was used to prepare Solution B7.

Solution B7 preparation

[0057] Solution B7 was prepared by weighing 2.5 mg of protamine sulfate into a 25 ml volumetric flask. Approximately 20 ml of phenol stock solution was added to the flask to dissolve the protamine sulfate. The q.s. amount of phenol stock solution was added to the flask. Solution B7 was filtered through a 0.22 µ filter into a 50 ml glass vial. Solution B7 contained phenol 4.4 mg/ml and protamine base 0.075 mg/ml in PBS.

Aqueous Suspension 7

[0058] 1.25 ml of solution A7 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.25 ml of solution B7 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 7 contained insulinotropin 1 mg/ml, phenol 2.2 mg/ml, and protamine base 0.038 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 8

Insulinotropin (1 mg/ml) Suspension

Solution A12 preparation

[0059] 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A12 was filtered by a syringe through a 0.22 µ filter into a 10 ml glass vial. Solution A12 contained insulinotropin 2 mg/ml in PBS.

Solution B12

[0060] Solution B12 was prepared by weighing 20 mg of phenol into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask. Solution B12 was filtered through a 0.22 µ filter into a 10 ml glass vial. Solution B12 contained phenol 2 mg/ml in PBS.

Aqueous Suspension 12

[0061] 4 ml of solution A12 was pipetted into a 10 ml type I glass vial. The contents of the vial were stirred while 4 ml of solution B12 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 12 contained insulinotropin 1 mg/ml and phenol 1 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 9

Insulinotropin (1 mg/ml) Suspension

Solution A15 preparation

[0062] 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of phosphate buffer (PB) was added to the flask to dissolve the drug. The q.s. amount of PB was added to the flask. Solution A15 was filtered by a syringe through a 0.22 μ filter into a 10 ml glass vial. Solution A15 contained insulinotropin 2 mg/ml in PB.

Solution B15 preparation

[0063] Solution B15 was prepared by weighing 8 mg of protamine sulfate into a 10 ml volumetric flask. Approximately 8 ml of PB was added to the flask to dissolve the protamine sulfate. The q.s. amount of PB was added to the flask. Solution B15 was filtered through a 0.22 μ filter into a 10 ml glass vial. Solution B15 contained protamine base 0.6 mg/ml in PBS.

Aqueous Suspension 15

[0064] 3 ml of solution A15 was pipetted into a 10 ml type I glass vial. The contents of the vial were stirred while 3 ml of solution B15 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 15 contained insulinotropin 1 mg/ml and protamine base 0.3 mg/ml in PB. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 10

Insulinotropin (1 mg/ml) Suspension

Solution A16 preparation

[0065] 20 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PB was added to the flask to dissolve the drug. The q.s. amount of PB was added to the flask. Solution A16 was filtered by a syringe through a 0.22 μ filter into a 10 ml glass vial. Solution A16 contained insulinotropin 2 mg/ml in PB.

Solution B16 preparation

[0066] Solution B16 was prepared by weighing 44 mg of phenol into a 10 ml volumetric flask. Approximately 8 ml of PB was added to the flask to dissolve the phenol. The q.s. amount of PB was added to the flask. Solution B16 was filtered through a 0.22 μ filter into a 10 ml glass vial. Solution B16 contained phenol 4.4 mg/ml in PB.

Aqueous Suspension 16

[0067] 3 ml of Solution A16 was pipetted into a 10 ml type I glass vial. The contents of the vial were stirred magnetically while 3 ml of Solution B16 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 16 contained insulinotropin 1 mg/ml and phenol 2.2 mg in PB. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 11

Insulinotropin (1 mg/ml) Suspension

Aqueous Suspension 17

[0068] 10 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. The contents of the flask was filtered by syringe through a 0.22 μ filter into a 10 ml type I glass vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 17 contained insulinotropin 1 mg/ml in PB. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 12

Insulinotropin (1 mg/ml) Suspension

Aqueous Suspension 18

[0069] 10 mg of insulinotropin was weighed into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. The contents of the flask were filtered by a syringe through a 0.22 μ filter into a 10 ml type I glass vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred gently (making sure no foam or bubble formed) for 16 hours to allow suspension formation. Aqueous Suspension 18 contained insulinotropin 1 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 13

Insulinotropin (0.2 mg/ml) Suspension

Solution A22 preparation

[0070] Solution A22 was prepared by weighing 2 mg of insulinotropin into a 5 ml volumetric flask. Approximately 3 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A22 was filtered by a syringe through a 0.22 μ filter into a 10 ml glass vial. Solution A22 contained insulinotropin 0.4 mg/ml in PBS.

Solution B22 preparation

[0071] Solution B22 was prepared by weighing 44 mg of phenol into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask. Solution B22 was filtered through a 0.22 μ filter into a 10 ml glass vial. Solution B22 contained phenol 4.4 mg/ml in PBS.

Aqueous Suspension 22

[0072] 1.5 ml of solution A22 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B22 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 22 contained insulinotropin 0.2 mg/ml and phenol 2.2 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 14

Insulinotropin (0.2 mg/ml) Suspension

Solution A23 preparation

[0073] Solution A23 was prepared by weighing 2 mg of insulinotropin into a 5 ml volumetric flask. Approximately 3 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A23 was filtered by a syringe through a 0.22 μ filter into a 10 ml glass vial. Solution A23 contained insulinotropin 0.4 mg/ml in PBS.

Solution B23 preparation

[0074] Solution B23 was prepared by weighing 8.8 mg of phenol into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask. Solution B23 was filtered through a 0.22 μ filter into a 10 ml glass vial. Solution B23 contained phenol 0.88 mg/ml in PBS.

Aqueous Suspension 23

[0075] 1.5 ml of solution A23 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred mag-

netically while 1.5 ml of solution B23 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 23 contained insulinotropin 0.2 mg/ml and phenol 0.44 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 15

Insulinotropin (1 mg/ml) Suspension

Solution A24 preparation

[0076] Solution A24 was prepared by weighing 10 mg of insulinotropin into a 5 ml volumetric flask. Approximately 3 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A24 was filtered by a syringe through a 0.22 μ filter into a 10 ml glass vial. Solution A24 contained insulinotropin 2 mg/ml in PBS.

Solution B24 preparation

[0077] Solution B24 was prepared by weighing 8 mg of protamine sulfate into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the protamine sulfate. The q.s. amount of PBS was added to the flask. Solution B24 was filtered through a 0.22 μ filter into a 10 ml glass vial. Solution B24 contained protamine base 0.6 mg/ml in PBS.

Aqueous Suspension 24

[0078] 1.5 ml of solution A24 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B24 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 24 contained insulinotropin 1 mg/ml and protamine base 0.3 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 16

Insulinotropin (1 mg/ml) Suspension

Solution A25 preparation

[0079] Solution A25 was prepared by weighing 10 mg of insulinotropin into a 5 ml volumetric flask. Approximately 3 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A25 was filtered by a syringe through a 0.22 μ filter into a 10 ml glass vial. Solution A25 contained insulinotropin 2 mg/ml in PBS.

Solution B25 preparation

[0080] Solution B25 was prepared by weighing 53 mg of m-cresol into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the m-cresol. The q.s. amount of PBS was added to the flask. Solution B25 was filtered through a 0.22 μ filter into a 10 ml glass vial. Solution B25 contained m-cresol 5.3 mg/ml in PBS.

Aqueous Suspension 25

[0081] 1.5 ml of solution A25 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B25 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 25 contained insulinotropin 1 mg/ml and m-cresol 2.5 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 17

Insulinotropin (0.5 mg/ml) Suspension

Solution A29 preparation

[0082] Solution A29 was prepared by weighing 25 mg of insulinotropin into a 25 ml volumetric flask. Approximately 20 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A29 was filtered by a syringe through a 0.22 µ filter into a 50 ml glass vial. Solution A29 contained insulinotropin 1 mg/ml in PBS.

Solution B29 preparation

[0083] Solution B29 was prepared by weighing 50 mg of phenol into a 50 ml volumetric flask. Approximately 40 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask. Solution B29 was filtered through a 0.22 µ filter into a 50 ml glass vial. Solution B29 contained phenol 1.0 mg/ml in PBS.

Aqueous Suspension 29

[0084] 1.5 ml of solution A29 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B29 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 29 contained insulinotropin 0.5 mg/ml and phenol 0.5 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 18

Insulinotropin (1 mg/ml) Suspension

Solution A31 preparation

[0085] 10 mg of insulinotropin was weighed into a 5 ml volumetric flask. Approximately 4 ml of PBS was added to the flask to disperse and dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A31 was filtered by a syringe through a 0.22 µ filter into a 10 ml glass vial. Solution A31 contained insulinotropin 2 mg/ml in PBS.

Solution B31 preparation

[0086] Solution B31 was prepared by weighing 50 mg of phenol into a 50 ml volumetric flask. Approximately 40 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask. Solution B31 was filtered through a 0.22 µ filter into a 50 ml glass vial. Solution B31 contained phenol 1 mg/ml in PBS.

Aqueous Suspension 31

[0087] 1.5 ml of solution A31 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B31 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred for 16 hours to allow suspension formation. Aqueous Suspension 31 contained insulinotropin 1 mg/ml and phenol 0.5 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 19

Insulinotropin (4 mg/mL) Suspension

Solution A51 preparation

[0088] 22 2 mg of insulinotropin was weighed into a 10 mL glass vial. 5 mL of PBS was pipetted into the vial to dissolve the drug. This solution was filtered through a 0.22 µ filter (low protein binding) into a 10 mL glass vial. Solution A51 contained insulinotropin 4.44 mg/mL in PBS.

Solution B51 preparation

[0089] 110 mg of phenol and 30 mg of protamine sulfate were weighed into a 5 mL volumetric flask. Approximately 4 mL of PBS was added to the flask to dissolve the phenol and protamine sulfate. The flask was filled to the mark with PBS. The solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution B51 contained phenol 22 mg/mL and protamine base 4.5 mg/mL in PBS.

Aqueous Suspension 51

[0090] 3 mL of Solution A51 and 0.33 mL of Solution B51 were pipetted into a 3.5 mL type I glass vial. The contents of the vial were shaken gently to ensure a homogeneous mix. The vial was allowed to sit at ambient temperature for 16 hours. Aqueous Suspension 51 contained insulinotropin 4 mg/mL, protamine base 0.44 mg/mL, and phenol 2.2 mg/mL in PBS. This suspension was used for in vivo pharmacokinetic studies in rats

EXAMPLE 20

Insulinotropin (4 mg/mL) Suspension

Solution A52 preparation

[0091] 22.2 mg of insulinotropin was weighed into a 10 mL glass vial. 5 mL of PBS was pipetted into the vial to dissolve the drug. This solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution A52 contained insulinotropin 4.44 mg/mL in PBS.

Solution B52 preparation

[0092] 110 mg of phenol and 15.6 mg of zinc acetate dihydrate were weighed into a 5 mL volumetric flask. Approximately 4 mL of water for injection was added to the flask to dissolve the phenol and zinc acetate dihydrate. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution B52 contained phenol 22 mg/mL and zinc acetate dihydrate 7.8 mg/mL in water for injection.

Aqueous Suspension 52

[0093] 3 mL of Solution A52 and 0.33 mL of Solution B52 were pipetted into a 3.5 mL type I glass vial. The contents of the vial were shaken gently to ensure a homogeneous mix. The vial was allowed to sit at ambient temperature for 16 hours. Aqueous Suspension 52 contained insulinotropin 4 mg/mL, zinc acetate dihydrate 0.78 mg/mL, and phenol 2.2 mg/mL in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 21

Insulinotropin (4 mg/mL) Suspension

Phenol Solution preparation

[0094] 244 mg of phenol was weighed into a 100 mL volumetric flask. Approximately 90 mL of water for injection was added to the flask to dissolve the phenol. The flask was filled to the mark with water for injection. The pH of this solution was adjusted to pH 9.0 with 5% NaOH solution. The Phenol Solution contained phenol 2.44 mg/mL in water for injection pH 9.0.

Solution A71 preparation

[0095] 22.2 mg of insulinotropin was weighed into a 10 mL glass vial. 5 mL of the Phenol Solution was pipetted into the vial to dissolve the drug. This solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution A71 contained insulinotropin 4.44 mg/mL and phenol 2.44 mg/mL in water for injection.

Solution B71 preparation

[0096] 116 mg of protamine sulfate was weighed into a 10 mL volumetric flask. Approximately 8 mL of water for

injection was added to the flask to dissolve the protamine sulfate. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 µ filter (low protein binding) into a 10 mL glass vial. Solution B71 contained protamine base 8.7 mg/mL in water for injection.

Solution C71 preparation

[0097] 156 mg of zinc acetate dihydrate and 1.632 g of NaCl were weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate and NaCl. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 µ filter (low protein binding) into a 10 mL glass vial. Solution C71 contained zinc acetate dihydrate 15.6 mg/mL and NaCl 163.2 mg/mL in water for injection.

Aqueous Suspension 71

[0098] 3 mL of Solution A71, 0.165 mL of Solution B71, and 0.165 mL of Solution C71 were pipetted into a 3.5 mL type I glass vial. The contents of the vial were shaken gently to ensure a homogeneous mix. The vial was allowed to sit at ambient temperature for 16 hours. Aqueous Suspension 71 contained insulinotropin 4 mg/mL, protamine base 0.435 mg/mL, zinc acetate dihydrate 0.78 mg/mL, NaCl 8.16 mg/mL, and phenol 2.2 mg/mL in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 22

Insulinotropin (4 mg/mL) Suspension

m-Cresol Solution preparation

[0099] 244 mg of m-cresol was weighed into a 100 mL volumetric flask. Approximately 90 mL of water for injection was added to the flask to dissolve the m-cresol. The flask was filled to the mark with water for injection. The pH of this solution was adjusted to pH 9.0 with 5% NaOH solution. The m-cresol Solution contained m-cresol 2.44 mg/mL in water for injection pH 9.0.

Solution A100 preparation

[0100] 22.2 mg of insulinotropin was weighed into a 10 mL glass vial. 5 mL of the m-cresol Solution was pipetted into the vial to dissolve the drug. This solution was filtered through a 0.22 µ filter (low protein binding) into a 10 mL glass vial. Solution A100 contained insulinotropin 4.44 mg/mL and m-cresol 2.44 mg/mL in water for injection.

Solution B100 preparation

[0101] 116 mg of protamine sulfate was weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the protamine sulfate. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 µ filter (low protein binding) into a 10 mL glass vial. Solution B100 contained protamine base 8.7 mg/mL in water for injection.

Solution C100 preparation

[0102] 156 mg of zinc acetate dihydrate and 1.632 g of NaCl were weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate and NaCl. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 µ filter (low protein binding) into a 10 mL glass vial. Solution C100 contained zinc acetate dihydrate 15.6 mg/mL and NaCl 163.2 mg/mL in water for injection.

Aqueous Suspension 100

[0103] 3 mL of Solution A100, 0.165 mL of Solution B100, and 0.165 mL of Solution C100 were pipetted into a 3.5 mL type I glass vial. The contents of the vial were shaken gently to ensure a homogeneous mix. The vial was allowed to sit at ambient temperature for 16 hours. Aqueous Suspension 100 contained insulinotropin 4 mg/mL, protamine base 0.435 mg/mL, zinc acetate dihydrate 0.78 mg/mL, NaCl 8.16 mg/mL, and m-cresol 2.2 mg/mL in water for injection.

This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 23

Insulinotropin (4 mg/mL) Suspension

Solution A68 preparation

[0104] 22.2 mg of insulinotropin was weighed into a 10 mL glass vial. 5 mL of the PBS was pipetted into the vial to dissolve the drug. This solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution A68 contained insulinotropin 4.44 mg/mL in PBS.

Solution B68 preparation

[0105] 116 mg of protamine sulfate was weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the protamine sulfate. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution B68 contained protamine base 8.7 mg/mL in water for injection.

Solution C68 preparation

[0106] 156 mg of zinc acetate dihydrate and 440 mg of phenol was weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate and phenol. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution C68 contained zinc acetate dihydrate 15.6 mg/mL and phenol 44 mg/mL in water for injection.

Aqueous Suspension 68

[0107] 3 mL of Solution A68, 0.165 mL of Solution B68, and 0.165 mL of Solution C68 were pipetted into a 3.5 mL type I glass vial. The contents of the vial were shaken gently to ensure a homogeneous mix. The vial was allowed to sit at ambient temperature for 16 hours. Aqueous Suspension 68 contained insulinotropin 4 mg/mL, protamine base 0.435 mg/mL, zinc acetate dihydrate 0.78 mg/mL, and phenol 2.2 mg/mL in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 24

Insulinotropin (4 mg/mL) Suspension

Solution A67 preparation

[0108] 22.2 mg of insulinotropin was weighed into a 10 mL glass vial. 5 mL of the PBS was pipetted into the vial to dissolve the drug. This solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution A67 contained insulinotropin 4.44 mg/mL in PBS.

Solution B67 preparation

[0109] 116 mg of protamine sulfate was weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the protamine sulfate. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution B67 contained protamine base 8.7 mg/mL in water for injection.

Solution C67 preparation

[0110] 156 mg of zinc acetate dihydrate and 440 mg of m-cresol were weighed into a 10 mL volumetric flask. Approximately 8 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate and m-cresol. The flask was filled to the mark with water for injection. The solution was filtered through a 0.22 μ filter (low protein binding) into a 10 mL glass vial. Solution C67 contained zinc acetate dihydrate 15.6 mg/mL and m-cresol 44 mg/mL in water

for injection.

Aqueous Suspension 67

[0111] 3 ml of Solution A67, 0.165 mL of Solution B67 and 0.165 mL of Solution C67 were pipetted into a 3.5 mL type I glass vial. The contents of the vial were shaken gently to ensure a homogeneous mix. The vial was allowed to sit at ambient temperature for 16 hours. Aqueous Suspension 67 contained insulinotropin 4 mg/mL, protamine base 0.435 mg/mL, zinc acetate dihydrate 0.75 mg/mL, and 1 m-cresol 2.2 mg/mL in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 25

Solution A39 preparation

[0112] 67.6 mg of insulinotropin was weighed into a glass vial. Approximately 22 mL of water for injection was added to the vial to dissolve the insulinotropin. The pH of the vial content was adjusted to 9.6 using NaOH to make a clear solution. Water for injection was added to the vial to make the final drug concentration to be 2.5 mg/ml.

Solution B39 preparation

[0113] 386.8 mg of zinc acetate dihydrate was weighed into a 100 ml volumetric flask. Approximately 80 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water for injection. Solution B39 contained zinc acetate dihydrate 3.9 mg/mL in water for injection.

Solution C39 preparation

[0114] 1.095 g of phenol was weighed into a 50 ml volumetric flask. Approximately 40 mL of water for injection was added to the flask to dissolve the phenol. The flask was filled to the mark with water for injection. Solution C39 contained phenol 21.9 mg/mL in water for injection.

Solution D39 preparation

[0115] 2.25 g of NaCl was weighed into a 25 mL volumetric flask. Approximately 20 mL of Solution C39 was added to the flask to dissolve the NaCl. The flask was filled to the mark with Solution C39. Solution D39 contained NaCl 9% (w/v) and phenol 21.9 mg/mL in water for injection.

Aqueous Suspension 39

[0116] All solutions were filtered through 0.22 µ filters (low protein binding). 9 ml of Solution A39 was transferred to a 10 ml sample vial. 1 ml of Solution B39 was added to the vial while stirring gently. Precipitates were formed immediately. The pH was measured to be 7.0. The vial was allowed to sit at ambient temperature for about 18 hours. 4 ml of the sample was transferred to a separate 10 ml vial, and 0.44 ml of Solution D39 was added to the vial. The sample was stirred gently for 5 minutes and was then allowed to sit at ambient temperature overnight.

[0117] Aqueous Suspension 39 contained insulinotropin 2 mg/ml, phenol 2.2 mg/ml, NaCl 0.9%, and zinc acetate 0.39 mg/ml. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 26

Solution A53 preparation

[0118] 32.5 mg of insulinotropin was weighed into a 10 ml glass vial. 6 ml of water for injection was added to the vial. The pH of the vial content was adjusted to 9.6 using 1 % (w/v) NaOH to make a clear solution. Appropriate amount of water for injection was added to make the drug concentration to be 5.0 mg/ml.

Solution B53 preparation

[0119] 390 mg of zinc acetate dihydrate was weighed into a 50 ml volumetric flask. Approximately 40 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water

for injection. Solution B53 contained zinc acetate dihydrate 7.8 mg/mL in water for injection.

Aqueous suspension 53

[0120] All solutions were filtered through 0.22μ filters (low protein binding). 2.4 mL of Solution A53 was transferred to a 3.5 ml vial. 300 μl of Solution B53 was added to the vial while stirring gently. Birefringent precipitates were formed immediately after the addition. The pH was measured to be 6.8. After the vial was allowed to sit at ambient temperature for 20 hours, 7.5 ul of m-cresol was added directly to the supernatant of the settled suspension. The suspension was then stirred gently to dissolve the m-cresol. 300 μl of 9% NaCl solution was added to the suspension with stirring. Aqueous Suspension 53 contained insulinotropin 4 mg/mL, 0.9% NaCl, 0.78 mg/mL zinc acetate, and 2.5 mg/mL m-cresol in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 27

Solution A54 preparation

[0121] 32.5 mg of insulinotropin was weighed into a 10 ml glass vial. 6 ml of water for injection was added to the vial. The pH of the vial content was adjusted to 9.6 using 1% (w/v) NaOH to make a clear solution. Appropriate amount of water for injection was added to make the drug concentration to be 5.0 mg/ml.

Solution B54 preparation

[0122] 390 mg of zinc acetate dihydrate was weighed into a 50 ml volumetric flask. Approximately 40 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water for injection. Solution B54 contained zinc acetate dihydrate 7.8 mg/mL in water for injection.

Solution C54 preparation

[0123] 1.1 g of phenol and 4.5 g of NaCl were weighed into a 50 ml volumetric flask. Approximately 40 mL of water for injection. The flask was filled to the mark with water for injection. Solution C54 contained phenol 22 mg/mL and NaCl 90 mg/mL.

Aqueous Suspension 54

[0124] All solutions were filtered through 0.22 μ filters (low protein binding). 2.4 ml of Solution A54 was transferred to a 3.5 ml vial. 300 μl of Solution B54 was added to the vial with stirring. Birefringent precipitates were formed immediately after the addition. The pH was measured to be 6.8. The sample was allowed to sit for 20 hours at ambient temperature. 300 μl of Solution C54 was added with gentle stirring. Aqueous Suspension 54 contained insulinotropin 4 mg/mL, zinc acetate dihydrate 0.78 mg/mL, phenol 2.2 mg/mL, and NaCl 9 mg/mL in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 28

Solution A57 preparation

[0125] 15 mg of insulinotropin was weighed into a 10 mL glass vial. 3 mL of water for injection was added to the vial. The pH of the vial content was adjusted to 9.9 using 5% NaOH to dissolve the drug completely. Solution A57 contained insulinotropin 5.0 mg/mL in water for injection.

Solution B57 preparation

[0126] 780 mg of zinc acetate dihydrate was weighed into a 100 mL volumetric flask. Approximately 80 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water for injection. Solution B57 contained zinc acetate dihydrate 7.8 mg/mL in water for injection.

Solution C57 preparation

[0127] 2.2 g of phenol and 9 g of NaCl were weighed into a 100 mL volumetric flask. Approximately 80 mL of water

for injection was added to the flask to dissolve the phenol and the NaCl. The flask was filled to the mark with water for injection. Solution C57 contained phenol 22 mg/ml and NaCl 90 mg/mL in water for injection.

Aqueous Suspension 57

[0128] 2.4 mL of Solution A57 was transferred to a 3.5 mL vial. The solution was stirred gently during addition of 300 µL of Solution B57. Precipitates were formed immediately after the addition of the Solution B57. The pH was measured and found to be 7.1. The sample was allowed to sit under ambient conditions for 24 hours. 300 µL of Solution C57 was added with gentle stirring. Aqueous Suspension 57 contained insulinotropin 4 mg/mL, zinc acetate dihydrate 0.78 mg/mL, phenol 2.2 mg/mL, and NaCl 9 mg/mL in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 29

Solution A64 preparation

[0129] 53.3 mg of insulinotropin was weighed into a 30 mL glass vial. After adding 11 mL of water for injection, the pH of the vial contents was adjusted to 8.3 using 5% NaOH (w/v) to dissolve the insulinotropin. The pH was adjusted down to 6.0 using dilute HCl making sure that the solution still remained clear. Appropriate amount of water for injection was added to make the drug concentration to be 4.4 mg/ml. Solution A64 was filtered through a 0.22 µ filter (low protein binding) into a 3.5 mL sample vial. 1.8 mL of the filtered solution was transferred to a separate sterile 3.5 mL vial, and the vial was allowed to sit at ambient temperature to crystallize for 3 days.

Solution B64 preparation

[0130] 780 mg of zinc acetate dihydrate was weighed into a 50 mL volumetric flask. Approximately 40 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water for injection. Solution B64 contained zinc acetate dihydrate 15.6 mg/mL in water for injection.

Solution C64 preparation

[0131] 18 g of NaCl was weighed into a 100 mL volumetric flask. Approximately 80 mL of water for injection was added to the flask to dissolve the NaCl. The flask was filled to the mark with water for injection. Solution C64 contained NaCl 180 mg/mL in water for injection.

Aqueous Suspension 64

[0132] After crystallization was completed in Solution A64, 100 µL of Solution B64 was added to 1.8 mL of the crystal suspension was slow stirring. The sample was then allowed to sit at ambient temperature for 3 days. 100 µL of Solution C64 was added to the crystal suspension with gentle stirring. The pH of the suspension was adjusted to pH 7.3 using dilute NaOH. 5.0 µ of m-cresol was added directly to the pH adjusted crystal suspension. Aqueous Suspension 64 contained insulinotropin 4 mg/mL, zinc acetate dihydrate 0.78 mg/mL, NaCl 9 mg/mL, and m-cresol 2.5 mg/mL in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 30

Solution A69 preparation

[0133] 1 g of NaCl was weighed into a 100 mL volumetric flask. Approximately 80 mL of water for injection was added to the flask to dissolve the NaCl. The flask was filled to the mark with water for injection. Solution A69 contained NaCl 1% (w/v) in water for injection.

Solution B69 preparation

[0134] 390 mg of zinc acetate dihydrate was weighed into a 100 mL volumetric flask. Approximately 80 mL of water for injection was added to the flask to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water for injection. Solution B69 contained zinc acetate dihydrate 3.9 mg/mL in water for injection.

Emulsion C69 preparation

[0135] 2.5 mL of sterile filtered (0.22 μ low protein binding) m-cresol was transferred to a 100 mL volumetric flask. The flask was filled with water for injection to the mark and sonicated to produce a homogenous suspension. Emulsion C69 contained m-cresol 25 mg/mL in water for injection.

Aqueous Suspension 69

[0136] 35.74 mg of insulinotropin was weighed into a 10 mL glass vial. 7 mL of Solution A69 was added. The pH of the vial contents was adjusted to 9.2 to dissolve the drug. The pH of the solution was re-adjusted to 6.5 using dilute HCl. Appropriate amount of water for injection was added to make the drug concentration to be 4.4 mg/ml. The solution was filtered through a 0.22 μ filter (low protein binding). The solution was allowed to sit at ambient temperature for 6 days, during which insulinotropin was crystallized. 1.5 mL of the crystal suspension was transferred to a separate vial. 167 μL of Solution B69 was added with gentle stirring. The sample was allowed to sit at ambient temperature for 1 day. 167 μL of emulsion C69 was added to the supernatant of the settled suspension. The sample was stirred to dissolve the m-cresol. Aqueous Suspension 69 contained insulinotropin 3.6 mg/ml, zinc acetate 0.36 mg/ml, NaCl 8.17 mg/ml and m-cresol 2.28 mg/ml in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 31

Solution A101 preparation

[0137] 10 g of sodium acetate was weighed into a 100 ml volumetric flask. Approximately 80 mL of water for injection was added to the flask to dissolve the sodium acetate. The flask was filled to the mark with water for injection. Solution A200 contained 100 mg/ml sodium acetate in water for injection.

Aqueous Suspension 101

[0138] 44.4 mg of insulinotropin was weighed into a 10 ml glass vial. 8 ml of water for injection was added to the flask. The pH of the vial contents was adjusted to 9.3 to obtain a clear solution. 1 mL of Solution A200 was added to the insulinotropin solution. The pH was then adjusted down to 6.5. The solution was filtered through a 0.22 μ filter (low protein binding). The filtered solution was allowed to sit at ambient temperature for 3 days so that crystallization could occur. Aqueous Suspension 101 contained insulinotropin 4.9 mg/mL sodium acetate 11.1 mg/mL in water for injection. This suspension was used for in vivo pharmacokinetic study in rats.

EXAMPLE 32

Solution A82 preparation

[0139] 9 g of NaCl was weighed into a 100 mL volumetric flask. Approximately 80 mL of water for injection was added to the vial to dissolve the NaCl. The flask was filled to the mark with water for injection. Solution A82 contained NaCl 9% (w/v) in water for injection.

Solution B82 preparation

[0140] 789 mg of zinc acetate dihydrate was weighed into a 100 mL volumetric flask. Approximately 80 mL of water for injection was added to the vial to dissolve the zinc acetate dihydrate. The flask was filled to the mark with water for injection. Solution B82 contained zinc acetate dihydrate 7.89 mg/mL in water for injection.

Emulsion C82 preparation

[0141] 2.5 mL of sterile filtered (0.22 μ low protein binding) m-cresol was transferred to a 100 mL volumetric flask. The flask was filled with water for injection to the mark and sonicated to produce a homogenous suspension. Emulsion C82 contained m-cresol 25 mg/mL in water for injection.

Aqueous Suspension 82

[0142] All solutions were filtered through 0.22 µ filters (low protein binding). 45.34 mg of insulinotropin was added to a 10 ml vial to which 8 ml of water was added. The pH was adjusted to 9.3 using 5% NaOH. After 1 ml of Solution A82 was added to the vial, the pH of the solution was adjusted down to 6.55 using dilute HCl. The solution (5 mg/mL insulinotropin) was filtered through a 0.22 µ filter (low protein binding). 81 µl of Aqueous Suspension 101 (see example 31) was added to the sterile filtered insulinotropin solution and dispersed by shaking the sample. The sample was then allowed to sit for 72 hours at ambient temperature to form a crystal suspension. 2.4 ml of the suspension was transferred to a 3.5 ml vial. 300 µl of Solution B82 was added to the vial with gentle stirring. The pH of the vial content was adjusted to 7.3 using dilute NaOH. 300 µl of Emulsion C82 was added to the supernatant of the settled suspension. Aqueous Suspension 82 contained insulinotropin 4 mg/ml, zinc acetate dihydrate 0.79 mg/mL, m-cresol 2.5 mg/mL and 0.9% NaCl in water for injection. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 33

GLP-1(7-36) Amide (1 mg/ml) Suspension

Solution A26 preparation

[0143] Solution A26 was prepared by weighing 10 mg of GLP-1 (7-36) Amide into a 5 ml volumetric flask. Approximately 3 ml of PBS was added to the flask to dissolve the drug. The q.s. amount of PBS was added to the flask. Solution A26 was filtered through a 0.22 µ filter into a 10 ml glass vial. Solution A26 contained GLP-1(7-36) 2 mg/ml in PBS.

Solution B26 preparation

[0144] Solution B26 was prepared by weighing 44 mg of phenol into a 10 ml volumetric flask. Approximately 8 ml of PBS was added to the flask to dissolve the phenol. The q.s. amount of PBS was added to the flask. Solution B26 was filtered through a 0.22 µ filter into a 10 ml glass vial. Solution B26 contained phenol 4.4 mg/ml in PBS.

Aqueous Suspension 26

[0145] 1.5 ml of solution A26 was pipetted into a 3.5 ml type I glass vial. The contents of the vial were stirred magnetically while 1.5 ml of solution B26 was pipetted into the vial. The vial was stoppered and sealed with an aluminum shell. The vial contents were stirred gently (making sure no foam or bubble formed) for 18 hours to allow suspension formation. Aqueous Suspension 26 contained GLP-1 (7-36) Amide 1 mg/ml and phenol 2.2 mg/ml in PBS. This suspension was used for in vivo pharmacokinetic studies in rats.

EXAMPLE 34

[0146] In one form of the invention, a low solubility form of GLP-1 (7-37) is prepared by combining GLP-1 (7-37) at from 2-15 mg/ml in buffer at pH 7-8.5 with a solution of a metal ion salt to obtain solutions with from 1-8 mg/ml GLP-1(7-37) at molar ratios of about 1:1 to 270:1 zinc to GLP-1 (7-37). A heavy precipitate forms and is let stand overnight at room temperature. The solubility of GLP-1(7-37) in the metal ion solution varies with the metal employed. Subsequent measurement of the solubility of the GLP-1 (7-37) pellet in a non metal-containing solvent such as PBS or water shows that zinc, cobalt and nickel ions produce low solubility forms of GLP-1 (7-37)

Table 1

Ability of Various metal ion salts to produce low solubility GLP-1(7-37)

Metal ion salt	Solubility in metal sol'n	Solubility in PBS
Zn Acetate	0.04 µg/ml	0.04 µg/ml
Zn Chloride	0.04 µg/ml	0.03 µg/ml
Co Chloride	0.11 µg/ml	0.04 µg/ml
Ni Sulfate	0.14 µg/ml	0.07 µg/ml
Mn Chloride	0.23 µg/ml	1.64 µg/ml
Mg Chloride	1.75 µg/ml	no ppt.
Ca Chloride	1.98 µg/ml	no ppt.

Note: In each case, 100 µl of metal ion solution at 5 mM was added to 100 µl GLP-1(7-37) at 5 mg/ml, mixed and allowed to stand overnight. The insoluble pellet was removed by centrifugation. The concentration of GLP-1(7-37) remaining in the metal ion solution was measured. The pellet was resuspended in phosphate buffered saline (PBS), sonicated and allowed to stand overnight. Again insoluble material was pelleted and GLP-1(7-37) concentration measured.

EXAMPLE 35

[0147] Microcrystalline forms of GLP-1(7-37) can be obtained by mixing solutions of GLP-1 (7-37) in buffer pH 7-8.5 with certain combinations of salts and low molecular weight polyethylene glycols (PEG). Table 2 describes six specific sets of conditions to produce microcrystalline forms of GLP-1(7-37).

Table 2

Selected Reagents Yielding Microcrystals			
Reagent#	Salt	Buffer	Precipitant
1	none	none	0.4M K, Na tartrate
2	0.2M Na citrate	0.1M Tris pH 8.5	30% PEG 400
3	0.2M $MgCl_2$	0.1M HEPES pH 7.5	28% PEG 400
4	0.2M $MgCl_2$	0.1M HEPES pH 7.5	30% PEG 400
5	0.5 M K_2HPO_4	none	20% PEG 8000
6	none	none	30% PEG 1500

Note: GLP-1(7-37) stock at 5 mg/ml in 50mM Tris pH 8.1 was added 1:1 with reagent. Drops were viewed and scored for absence or presence of insoluble GLP-1 (7-37) in crystalline or amorphous form. In general low mw PEG's appear to favor crystalline forms. Tris is tris(hydroxymethyl)aminomethane and HEPES is N-2-(Hydroxyethyl) piperazine-N-2-ethanesulfonic acid.

COMPARATIVE EXAMPLE 36

[0148] Specific combinations of GLP-1(7-37) and PEG concentrations are required to obtain microcrystalline forms and high yields. Table 3 shows specific combinations of PEG 600 and GLP-1(7-37) concentrations which produce microcrystalline as opposed to amorphous forms of the drug. The yield of GLP-1(7-37) in the insoluble form is shown also.

Table 3

Formation/yield of crystalline GLP-1 (7-37)			
GLP-1 (7-37)	15 PEG 600	22.5% PEG 600	30% PEG 600
2.0 mg/ml (Form/yield)	amorphous/8%	amorphous/10%	amorphous 8%
3.5 mg/ml (Form/yield)	crystalline/62%	crystalline/26%	crystalline/59%
5.0 mg/ml (Form/yield)	amorphous/34%	crystalline/63%	crystalline/72%
6.5 mg/ml (Form/yield)	amorphous/52%	crystalline/76%	crystalline/82%
8.0 mg/ml (Form/yield)	amorphous/55	crystalline/82%	amorphous/66%
9.5 mg/ml (Form/yield)	amorphous/69%	crystalline/85%	amorphous/83%

Note: Microcrystals of GLP-1(7-37) are prepared by combining solutions of GLP-1 (7-37) at 20 mg/ml in tris buffer at pH 8, 60% polyethylene glycol 600 (PEG 600) in H_2O and tris buffer pH 8 to obtain a final concentrations of from 15-30% PEG and from 3-10 mg/ml GLP-1. After standing overnight, microcrystals of GLP-1(7-37) form in the solution with yields from 50-85%.

EXAMPLE 37

[0149] This experiment exemplifies another form of the invention which involves treating preformed microcrystals of GLP-1(7-37) with various metal ions to produce low solubility microcrystalline forms. Microcrystals of GLP-1(7-37) prepared at 8 mg/ml GLP-1(7-37) and 22.5% PEG as described in Example 36 have a solubility equivalent to pure lyophilized GLP-1 (7-37). In order to impart the desired property of low solubility for long-acting drug delivery, these preformed microcrystals can be treated with solutions of metal salts at ratios of metal:GLP-1(7-37) of from 1:1 to 260:1 overnight at room temp. The excess metal salt was removed by a centrifugation/washing process. Table 4 shows the results with several divalent cation metal salts as treatment.

Table 4

Solubility of GLP-1(7-37) Crystals with Various Treatments			
Additive	GLP-1(7-37) (mg/ml) in treatment sol'n	GLP-1(7-37) (mg/ml) in PBS	GLP-1(7-37) (mg/ml) in PBS/EDTA
None (PBS)	1.2	1.2	ND
Citrate pH 5.2	0.15	ND	ND
$ZnCl_2$ pH 5.2	0.03	0.03	1.1
ZnAc pH 5.2	0.01	0.02	1.1
ZnAc pH 6.5	0.06	0.02	0.92
$MgSO_4$ pH 5.2	0.50	0.55	ND
$NiSO_4$ pH 5.2	0.10	0.04	0.45
$MnCl_2$ pH 5.2	0.10	0.10	ND
$CaCl_2$ pH 5.2	0.40	0.27	ND

Note: GLP-1(7-37) crystals are grown from a solution of 8 mg/ml IST in 50 mM Tris pH 8 with 22.5% PEG 600 added in H_2O. All additive treatment solutions are 100 mM divalent ion salt in 10 mM Na citrate pH 5.2 or Na MES pH 6.5.

EXAMPLE 38

[0150] Using the methods described herein, both amorphous and microcrystalline low solubility formulations were prepared using zinc acetate. Subcutaneous injections were made in rats (three animals per formulation) and plasma levels of GLP-1 (7-37) were measured by radioimmune assay over 24 hours. Figure 10 shows the extended duration of the drug in plasma compared to a subcutaneous control injection of soluble GLP-1 (7-37).

EXAMPLE 39

[0151] 45% w/v Polyethylene Glycol 3350 (PEG)
1 mg/ml Insulinotropin
20 mM Phosphate Buffer
qs Sterile Water for Injection (SWFI)

[0152] A 50% w/w PEG solution was prepared using SWFI. A 200 mM phosphate buffer was separately prepared with anhydrous sodium phosphate dibasic (26.85 mg/ml) and sodium phosphate monobasic monohydrate (1.41 mg/ml). If necessary, the pH of the buffer solution was brought to pH 8 with either sodium hydroxide or hydrochloric acid. The appropriate amount of insulinotropin was dissolved in enough of the buffer solution to make a 10 mg/ml solution of insulinotropin. The appropriate weight of the PEG solution was added to the insulinotropin solution, and a sufficient quantity of SWFI was used to bring the solution to the desired volume. The final solution was then sterile filtered with 0.2 μ filter and aseptically filled into vials. The solution (0.5 ml) was injected subcutaneously in rats, and plasma insulinotropin levels followed by RIA assay.

EXAMPLE 40

[0153] 1.32% w/v Hydroxyethyl Cellulose (HEC)
1 mg/ml Insulinotropin
20 mM Phosphate Buffer
100 mM Sodium Chloride
qs Sterile Water For Injection (SWFI)

[0154] A 2% w/w hydroxethyl cellulose solution was prepared using SWFI. A 200 mM phosphate buffer was separately prepared with anhydrous sodium phosphate dibasic (26.85 mg/ml) and sodium phosphate monobasic monohydrate (1.41 mg/ml). If necessary, the pH of the buffer solution was brought to pH 8 with either sodium hydroxide or hydrochloric acid. The appropriate amount of insulinotropin and sodium chloride were dissolved in enough of the buffer solution to make a 10 mg/ml solution of insulinotropin. The appropriate weight of the HEC solution was added to the insulinotropin solution, and a sufficient quantity of SWFI was used to bring the solution to the desired volume. The final solution was then sterile filtered with a 0.2 μ filter and aseptically filled into vials. The solution (0.5 ml) was injected subcutaneously in rats, and plasma insulinotropin followed by RIA assay.

EXAMPLE 41

[0155] 18% w/v Pluronic F127
1 mg/ml Insulinotropin
20 mM Phosphate Buffer
qs Sterile Water For Injection (SWFI)

[0156] A 20% W/W Pluronic F127 solution was prepared using SWFI. A Polytron (probe homogenizer) with an ice bath was used to dissolve the polymer. A 200 mM phosphate buffer was separately prepared with anhydrous sodium phosphate dibasic (26.85 mg/ml) and sodium phosphate monobasic monohydrate (1.41 mg/ml). If necessary, the pH of the buffer solution was brought to pH 8 with either sodium hydroxide or hydrochloric acid. The appropriate amount of insulinotropin was dissolve in enough of the buffer solution to make a 10 mg/ml solution of insulinotropin. The appropriate weight of the Pluronic solution was added to the insulinotropin solution, and a sufficient quantity of SWFI was used to bring the solution to the desired volume. The final solution was then sterile filtered with a 0.2 μm filter and aseptically filled into vials. The solution (0.5 ml) was injected subcutaneously in rats, and plasma insulinotropin levels followed by RIA assay.

EXAMPLE 42

[0157] Peanut Oil Suspension (Ball Milled)
1 mg/ml Insulinotropin

1% Tween 80

[0158] Tween 80 was added at 1% level to peanut oil. This solution was sterile filtered with a 0.2 µm filter. Solid insulinotropin was then suspended in the oil. The particle size was reduced by ball milling with a Szesvari Attritor at 40 RPM for 18 hours (cold water jacket). This suspension was then filled into vials. The suspension (0.5 ml) was injected subcutaneously in rats, and plasma insulinotropin levels followed by RIA assay.

EXAMPLE 43

[0159] 22.6% w/v Dextran
1 mg/ml Insulinotropin
20 mM Phosphate Buffer
qs Sterile Water for Injection

[0160] A 50% w/w Dextran solution was prepared using SWFI. A 200 mM phosphate buffer was separately prepared with anhydrous sodium phosphate dibasic (26.85 mg/ml) and sodium phosphate monobasic monohydrate (1.41 mg/ml). If necessary, the pH of the buffer solution was brought to pH 8 with either sodium hydroxide or hydrochloric acid. The appropriate amount of insulinotropin was dissolved in enough of the buffer solution to make 5.0 mg/ml solution of insulinotropin. The appropriate weight of the dextran solution was added to the insulinotropin solution, and a sufficient quantity of SWFI was used to bring the solution to the desired volume. The final solution was then sterile filtered with 0.2 µm filter and aseptically filled into vials. The solution (0.5 ml) was injected subcutaneously into rats, and plasma insulinotropin levels were followed by RIA assay.

EXAMPLE 44

[0161] Insulinotropin was crystallized from the mixture of phosphate buffered saline (PBS), ethanol, and m-cresol. A homogeneous insulinotropin slurry (10 mg/ml) was made with PBS in a glass vial, and a large volume of ethanol (9 times as much as the slurry) was added to the vial while the vial content was stirred magnetically. Very fine amorphous particles of insulinotropin formed. m-Cresol was added to the vial so that the resulting m-cresol concentration was 1% (v/v). The vial was capped to prevent solvent from evaporating. The crystallization mixture was stored at room temperature for a couple of days. Needle shape crystalline plates grew from the amorphous particles. The lengths of the crystals are between 50 and 200 µm, and widths between 2 and 4 µm.

EXAMPLE 45

[0162] Insulinotropin (1 to 4 mg/mL) was dissolved in 1% sodium sulfate (or sodium acetate, or sodium chloride, or ammonium sulfate) solution at higher pH values than 8, and the pH of the solution was lowered down to 6.0 to 7.5 with d-HCl. The clear solution was allowed to sit at ambient temperature. After a couple of days, needle or plate shape crystals were obtained depending on the crystallization conditions.

EXAMPLE 46

[0163] GLP-1(7-37) was dissolved in 50 mM glycine buffer containing 0.1 to 0.2 M NaCl at pH 8.5-9.5 at from 1 to 5 mg/ml. A solution of zinc salt (acetate or chloride) was added to obtain a molar ratio of from 0.5:1 to 1.5:1 zinc:GLP-1(7-37). Crystals of GLP-1(7-37) grew overnight at room temperature with yields from 70 to 97%.

EXAMPLE 47

[0164] GLP-1(7-37) crystals can be grown by vapor diffusion using the peptide dissolved in 100 mM Tris at pH 8-9.5 at from 10-20 mg/ml. The peptide solution is mixed 1:1 with the same buffer containing from 0.5 to 2.5 M NaCl then equilibrated in a sealed system against the full strength buffer (i.e. Tris with 0.5 -2.5 M NaCl).

SEQUENCE LISTING

[0165]

(1) GENERAL INFORMATION:

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Lambert, William J.

Qi, Hong
Gelfand, Robert A.
Geoghegan, Kieran F.
Danley, Dennis E.

(ii) TITLE OF INVENTION: Prolonged Delivery of Peptides

(iii) NUMBER OF SEQUENCES: 7

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(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10017-5755

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Sheyka, Robert F.
(B) REGISTRATION NUMBER: 31,304
(C) REFERENCE/DOCKET NUMBER: PC8391

(ix) TELECOMMUNICATION INFORMATION:

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(C) TELEX: N/A

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A
(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A
(B) MAP POSITION: N/A
(C) UNITS: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val
1               5                  10                  15

Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu
            20                  25                  30

Val Lys Gly Arg Gly
        35
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A
(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A
(B) MAP POSITION: N/A
(C) UNITS: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A
(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A
(B) MAP POSITION: N/A
(C) UNITS: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A
(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A
(B) MAP POSITION: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly
            20                  25
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A
(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A
(B) MAP POSITION: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A

(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A
(B) MAP POSITION: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val
1               5                  10                  15

Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu
            20                  25                  30

Val Lys Gly Arg
        35
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: N/A
(B) STRAIN: N/A
(C) INDIVIDUAL ISOLATE: N/A
(E) HAPLOTYPE: N/A
(H) CELL LINE: N/A

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: N/A
(B) CLONE: N/A

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: N/A

(B) MAP POSITION: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
1               5                  10                  15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val
            20                  25
```

Claims

1. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a polymer selected from polyethylene glycol, polyoxyethylene-polyoxypropylene copolymers, polyanhydrides and polysaccharides, wherein said polysaccharides are selected from chitosan, acacia gum, karaya gum, guar gum, xanthan gum, tragacanth, alginic acid, carrageenan, agarose, and furcellarans, dextran, starch, starch derivatives and hyaluronic acid;

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and has been treated in such a way that it comprises said compound of part (i) in crystalline or amorphous form having a solubility equal to or less than 500 µg/ml under physiological conditions.

2. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE NO: 2, SEQUENCE ID NO: 3, SEQUENCE NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a pharmaceutically acceptable water-immiscible oil suspension; said oil selected peanut oil, sesame oil, almond oil, caster oil, camellia oil, cotton seed oil, olive oil, corn oil, soy oil, safflower oil, esters of fatty acids, and esters of fatty alcohols

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in particulate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

3. A composition according to claim 2, further comprising (i) a wetting agent which is a non-ionic surfactant and (i) a suspending agent.

4. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys.
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from :

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or and C_1-C_6 dialkyl amide of said peptides, and

(ii) zinc (II), which is mixed with the peptide;

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in crystalline or amorphous form having a solubility equal to or less than 500 µg/ml under physiological conditions.

5. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from :

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides,

and

(ii) a metal selected from Ni(II), Co(II), Mn(II), Fe(II), and Cu(II),

wherein said composition of matter is in an injectable formulation and comprises said compound of part (i) in crystalline or amorphous form having a solubility equal to or less than 500 µg/ml under physiological conditions.

6. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

H_2N-W-COOH

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

H_2N-R-COOH

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) phenol, cresol, resorcinol or methyl paraben,

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

7. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is :

(A) Lys,
(B) Lys-Gly. or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6:
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a basic polypeptide and a phenolic compound,

wherein said composition of matter is in an injectable formulation, is capable of achieving sustained glycaemic control and comprises said compound of part (i) in precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

8. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE NO: 3, SEQUENCE NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;

(4) a pharmaceutically acceptable alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a basic polypeptide, a phenolic compound, and a metal ion;

wherein said composition of matter is in injectable form, is capable of achieving sustained glycaemic control and comprises said compound of part (i) precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

9. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE ID NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE ID NO: 7;
wherein X is:

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE ID NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE ID NO: 2, SEQUENCE ID NO: 3, SEQUENCE ID NO: 4 or SEQUENCE ID NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable lower alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said eptides,

said peptides and derivatives thereof having being subjected to conditions resulting in amorphous or crystalline precipitates or aggregates having a solubility equal to or less than 500 µg/ml under physiological conditions; wherein said conditions are high shear, exposure to salts; or combinations thereof;

wherein said composition of matter is in an injectable formulation and is capable of achieving sustained glycaemic control.

10. A composition according to claim 9, wherein said salt is ammonium sulphate, sodium sulphate, lithium sulphate, lithium chloride, sodium citrate, ammonium citrate, sodium phosphate, potassium phosphate, sodium chloride, potassium chloride, ammonium chloride, sodium acetate, ammonium acetate, magnesium sulphate, calcium chloride, ammonium nitrate, sodium formate, or a combination thereof.

11. A composition of matter comprising:

(i) a compound selected from:

(a) a peptide having the amino acid sequence of SEQUENCE NO: 2;
(b) a peptide having the amino acid sequence of SEQUENCE NO: 7;
wherein X is :

(A) Lys,
(B) Lys-Gly, or
(C) Lys-Gly-Arg;

(c) a peptide comprising the primary structure

$$H_2N\text{-}W\text{-}COOH$$

wherein W is the amino acid sequence of SEQUENCE NO: 1 or SEQUENCE ID NO: 6;
(d) a peptide comprising the primary structure

$$H_2N\text{-}R\text{-}COOH$$

wherein R is the amino acid sequence of SEQUENCE NO: 2, SEQUENCE NO: 3, SEQUENCE ID NO: 4 or SEQUENCE NO: 5; and
(e) a derivative of said peptides (a) through (d) selected from:

(1) a pharmaceutically acceptable acid addition salt of said peptides;
(2) a pharmaceutically acceptable carboxylate salt of said peptides;
(3) a pharmaceutically acceptable alkali addition salt of said peptides;
(4) a pharmaceutically acceptable C_1-C_6 alkyl ester of said peptides; and
(5) a pharmaceutically acceptable amide, C_1-C_6 alkyl amide or C_1-C_6 dialkyl amide of said peptides, and

(ii) a basic polypeptide;

wherein said composition of matter is in injectable form, is capable of achieving sustained glycaemic control and comprises said compound of part (i) precipitate or aggregate form having a solubility equal to or less than 500 µg/ml under physiological conditions.

12. A composition according to any of claims 1 to 11 for use in medicine.

13. The use of a composition according to any of claims 1 to 11 in the manufacture of a medicament for treating non-insulin dependent diabetes mellitus.

Patentansprüche

1. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;

(d) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-R-COOH

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und

(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1)einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2)einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4)einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder Ci-C_6-Dialkylamid der Peptide, und

(ii) ein Polymer ausgewählt aus Polyethylenglykol, Polyoxyethylen-Polyoxypropylen-Copolymeren, Polyanhydriden und Polysacchariden, wobei die Polysaccharide aus Chitosan, Gummiarabikum, Karayagummi, Guar Gum, Xanthan, Tragant, Alginsäure, Carrageenan, Agarose, und Furcellaranen, Dextran, Stärke, Stärkederivaten und Hyaluronsäure ausgewählt sind,

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und in einer Weise behandelt worden ist, dass sie die Verbindung aus Teil (i) in kristalliner oder amorpher Form aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

2. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;

(d) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-R-COOH

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und

(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;

(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) eine pharmazeutisch akzeptable, nicht mit Wasser mischbare Ölsuspension, wobei das Öl aus Erdnussöl, Sesamöl, Mandelöl, Rizinusöl, Kamelienöl, Baumwollsamenöl, Olivenöl, Maisöl, Sojaöl, Safloröl, Estern von Fettsäuren und Estern von Fettalkoholen ausgewählt ist,

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und die Verbindung aus Teil (i) in Partikelform aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

3. Zusammensetzung nach Anspruch 2, welche ferner (i) ein Benetzungsmittel, bei welchem es sich um einen nichtionischen oberflächenaktiven Stoff handelt, und (ii) ein Suspendiermittel aufweist.

4. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

$$H_2N\text{-}W\text{-}COOH$$

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;
(d) einem Peptid, welches folgende Primärstruktur aufweist:

$$H_2N\text{-}R\text{-}COOH$$

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und
(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) Zink (II), welches mit dem Peptid gemischt ist,

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und die Verbindung aus Teil (i) in kristalliner oder amorpher Form aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

5. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;
(d) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-R-COOH

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und
(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) ein Metall ausgewählt aus Ni(II), Co(II), Mn(II), Fe(II) und Cu(II),

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt und die Verbindung aus Teil (i) in kristalliner oder amorpher Form aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

6. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;
(d) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-R-COOH

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und

(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) Phenol, Cresol, Resorcinol oder Methylparaben,

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und die Verbindung aus Teil (i) in Präzipitat- oder Aggregatform aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

7. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;

(d) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-R-COOH

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und

(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) ein basisches Polypeptid und eine Phenolverbindung,

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und die Verbindung aus Teil (i) in Präzipitat- oder Aggregatform aufweist, welche unter

physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

8. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;
(d) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-R-COOH

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und
(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) ein basisches Polypeptid, eine Phenolverbindung und ein Metall-Ion,

wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und die Verbindung aus Teil (i) in Präzipitat- oder Aggregatform aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

9. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

H_2N-W-COOH

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;
(d) einem Peptid, welches folgende Primärstruktur aufweist:

$$H_2N\text{-}R\text{-}COOH$$

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und
(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;
(4) einem pharmazeutisch akzeptablen Niederalkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide,

wobei die Peptide und Derivate davon Bedingungen unterzogen wurden, welche in amorphen oder kristallinen Präzipitaten oder Aggregaten resultieren, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweisen, wobei die Bedingungen folgende sind: hohe Scherung, Salzeinwirkung oder Kombinationen davon;
wobei die Stoffzusammensetzung in einer injizierbaren Form vorliegt und in der Lage ist, verzögerte glykämische Kontrolle zu erreichen.

10. Zusammensetzung nach Anspruch 9, wobei das Salz Ammoniumsulfat, Natriumsulfat, Lithiumsulfat, Lithiumchlorid, Natriumcitrat, Ammoniumcitrat, Natriumphosphat, Kaliumphosphat, Natriumchlorid, Kaliumchlorid, Ammoniumchlorid, Natriumacetat, Ammoniumacetat, Magnesiumsulfat, Calciumchlorid, Ammoniumnitrat, Natriumformat oder eine Kombination daraus ist.

11. Stoffzusammensetzung, welche Folgendes aufweist:

(i) eine Verbindung ausgewählt aus:

(a) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 2;
(b) einem Peptid mit der Aminosäuresequenz von SEQUENZ ID NR.: 7;
wobei X Folgendes ist:

(A) Lys,
(B) Lys-Gly, oder
(C) Lys-Gly-Arg;

(c) einem Peptid, welches folgende Primärstruktur aufweist:

$$H_2N\text{-}W\text{-}COOH$$

wobei W die Aminosäuresequenz von SEQUENZ ID NR.: 1 oder SEQUENZ ID NR.: 6 ist;
(d) einem Peptid, welches folgende Primärstruktur aufweist:

$$H_2N\text{-}R\text{-}COOH$$

wobei R die Aminosäuresequenz von SEQUENZ ID NR.: 2, SEQUENZ ID NR.: 3, SEQUENZ ID NR.: 4 oder SEQUENZ ID NR.: 5 ist; und
(e) einem Derivat der Peptide (a) bis (d) ausgewählt aus:

(1) einem pharmazeutisch akzeptablen Säureadditionssalz der Peptide;
(2) einem pharmazeutisch akzeptablen Carboxylatsalz der Peptide;
(3) einem pharmazeutisch akzeptablen Alkaliadditionssalz der Peptide;

(4) einem pharmazeutisch akzeptablen C_1-C_6-Alkylester der Peptide; und
(5) einem pharmazeutisch akzeptablen Amid, C_1-C_6-Alkylamid oder C_1-C_6-Dialkylamid der Peptide, und

(ii) ein basisches Polypeptid,

wobei die Stoffzusammensetzung in injizierbarer Form vorliegt, in der Lage ist, verzögerte glykämische Kontrolle zu erreichen und die Verbindung aus Teil (i) in Präzipitat- oder Aggregatform aufweist, welche unter physiologischen Bedingungen eine Löslichkeit gleich oder unter 500 µg/ml aufweist.

12. Zusammensetzung nach einem der Ansprüche 1 bis 11 zur Verwendung in der Medizin.

13. Verwendung einer Zusammensetzung nach einem der Ansprüche 1 bis 11 bei der Herstellung eines Medikamentes zur Behandlung von Nichtinsulinabhängigem Diabetes Mellitus.

Revendications

1. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

$$H_2N\text{-}W\text{-}COOH$$

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

$$H_2N\text{-}R\text{-}COOH$$

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) un polymère sélectionné parmi un polyéthylèneglycol, des copolymères de polyoxyéthylène-polyoxypropylène, des polyanhydrides et des polysaccharides, dans lesquels lesdits polysaccharides sont sélectionnés parmi du chitosan, de la gomme d'acacia, de la gomme karaya, de la gomme de guar, de la gomme de xanthane, de la gomme adragante, de l'acide alginique, de la carragénine, de l'agarose et des furcellaranes, du dextrane, de l'amidon, des dérivés d'amidon et de l'acide hyaluronique;

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et a été traitée d'une manière telle qu'elle comprend ledit composé de la partie (i) sous une forme cristalline ou amorphe présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

2. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

$$H_2N\text{-}W\text{-}COOH$$

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

$$H_2N\text{-}R\text{-}COOH$$

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) une suspension huileuse non miscible dans de l'eau pharmaceutiquement acceptable; ladite huile étant sélectionnée parmi de l'huile d'arachide, de l'huile de sésame, de l'huile d'amande, de l'huile de ricin, de l'huile de camélia, de l'huile de graines de coton, de l'huile d'olive, de l'huile de maïs, de l'huile de soja, de l'huile de carthame, des esters d'acides gras et des esters d'alcools gras

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et comprend ledit composé de la partie (i) sous une forme particulaire présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

3. Composition suivant la revendication 2, comprenant en outre (i) un agent mouillant qui est un tensioactif non ionique et (i) un agent de mise en suspension.

4. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

H_2N-W-COOH

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

H_2N-R-COOH

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) du zinc (II), qui est en mélange avec le peptide;

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et comprend ledit composé de la partie (i) sous une forme cristalline ou amorphe présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

5. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

H_2N-W-COOH

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

H_2N-R-COOH

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO.3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) un métal sélectionné parmi du Ni(II), du Co(II), du Mn (II), du Fe (II) et du Cu(II),

dans laquelle ladite composition de matière est dans une formulation injectable et comprend ledit composé de la partie (i) sous une forme cristalline ou amorphe présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

6. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

$$H_2N\text{-}W\text{-}COOH$$

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

$$H_2N\text{-}R\text{-}COOH$$

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) du phénol, du crésol, du résorcinol ou du méthylparabène,

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et comprend ledit composé de la partie (i) sous une forme de précipité ou d'agrégat présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

7. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;

(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

H_2N-W-COOH

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

H_2N-R-COOH

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) un polypeptide basique et un composé phénolique,

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et comprend ledit composé de la partie (i) sous une forme de précipité ou d'agrégat présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

8. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

H_2N-W-COOH

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

H_2N-R-COOH

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;.
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) un polypeptide basique, un composé phénolique et un ion métallique;

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et comprend ledit composé de la partie (i) sous une forme de précipité ou d'agrégat présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

9. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

$$H_2N\text{-}W\text{-}COOH$$

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

$$H_2N\text{-}R\text{-}COOH$$

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle inférieur pharmaceutiquement acceptable desdits peptides;
et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

lesdits peptides et dérivés de ceux-ci ayant été soumis à des conditions donnant des précipités ou des agrégats amorphes ou cristallins présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques; dans laquelle lesdites conditions sont un haut cisaillement, une exposition à des sels; ou des combinaisons de ceux-ci;
dans laquelle ladite composition de matière est dans une formulation injectable et peut donner un contrôle glycémique prolongé.

10. Composition suivant la revendication 9, dans laquelle ledit sel est du sulfate d'ammonium, du sulfate de sodium, du sulfate de lithium, du chlorure de lithium, du citrate de sodium, du citrate d'ammonium, du phosphate de sodium, du phosphate de potassium, du chlorure de sodium, du chlorure de potassium, du chlorure d'ammonium, de l'acétate de sodium, de l'acétate d'ammonium, du sulfate de magnésium, du chlorure de calcium, du nitrate d'ammonium, du formiate de sodium ou une combinaison de ceux-ci.

11. Composition de matière comprenant :

(i) un composé sélectionné parmi :

(a) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:2;
(b) un peptide présentant la séquence d'acides aminés de la SEQ ID NO:7;
dans laquelle X est :

(A) Lys,
(B) Lys-Gly, ou
(C) Lys-Gly-Arg;

(c) un peptide comprenant la structure primaire

H_2N-W-COOH

dans laquelle W est la séquence d'acides aminés de la SEQ ID NO:1 ou de la SEQ ID NO:6;
(d) un peptide comprenant la structure primaire

H_2N-R-COOH

dans laquelle R est la séquence d'acides aminés de la SEQ ID NO:2, de la SEQ ID NO:3, de la SEQ ID NO:4 ou de la SEQ ID NO:5; et
(e) un dérivé desdits peptides (a) à (d) sélectionné parmi :

(1) un sel d'addition d'acide pharmaceutiquement acceptable desdits peptides;
(2) un sel carboxylate pharmaceutiquement acceptable desdits peptides;
(3) un sel d'addition d'alcali pharmaceutiquement acceptable desdits peptides;
(4) un ester d'alkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides; et
(5) un amide, un amide d'alkyle en C_1-C_6 ou un amide de dialkyle en C_1-C_6 pharmaceutiquement acceptable desdits peptides, et

(ii) un polypeptide basique;

dans laquelle ladite composition de matière est dans une formulation injectable, peut donner un contrôle glycémique prolongé et comprend ledit composé de la partie (i) sous une forme de précipité ou d'agrégat présentant une solubilité égale ou inférieure à 500 µg/ml dans des conditions physiologiques.

12. Composition suivant l'une quelconque des revendications 1 à 11, pour une utilisation en médecine.

13. Utilisation d'une composition suivant l'une quelconque des revendications 1 à 11, dans la fabrication d'un médicament pour le traitement du diabète sucré non insulinodépendant.

FIG. 1

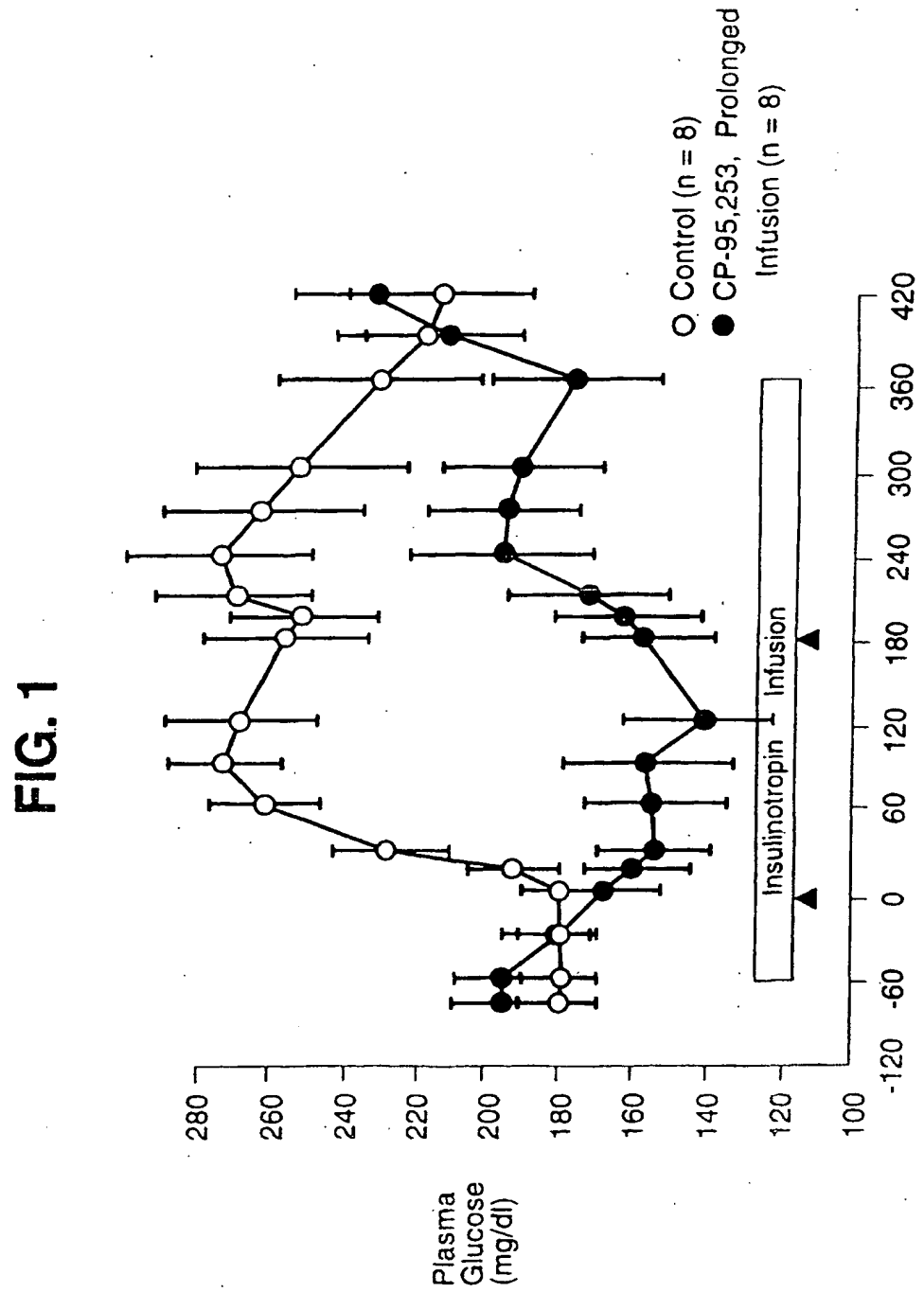

O Control (n = 8)
● CP-95,253, Prolonged Infusion (n = 8)
Insulinotropin Infusion
Plasma Glucose (mg/dl)
280
260
240
220
200
180
160
140
120
100
-120
-60
0
60
120
180
240
300
360
420

FIG. 2

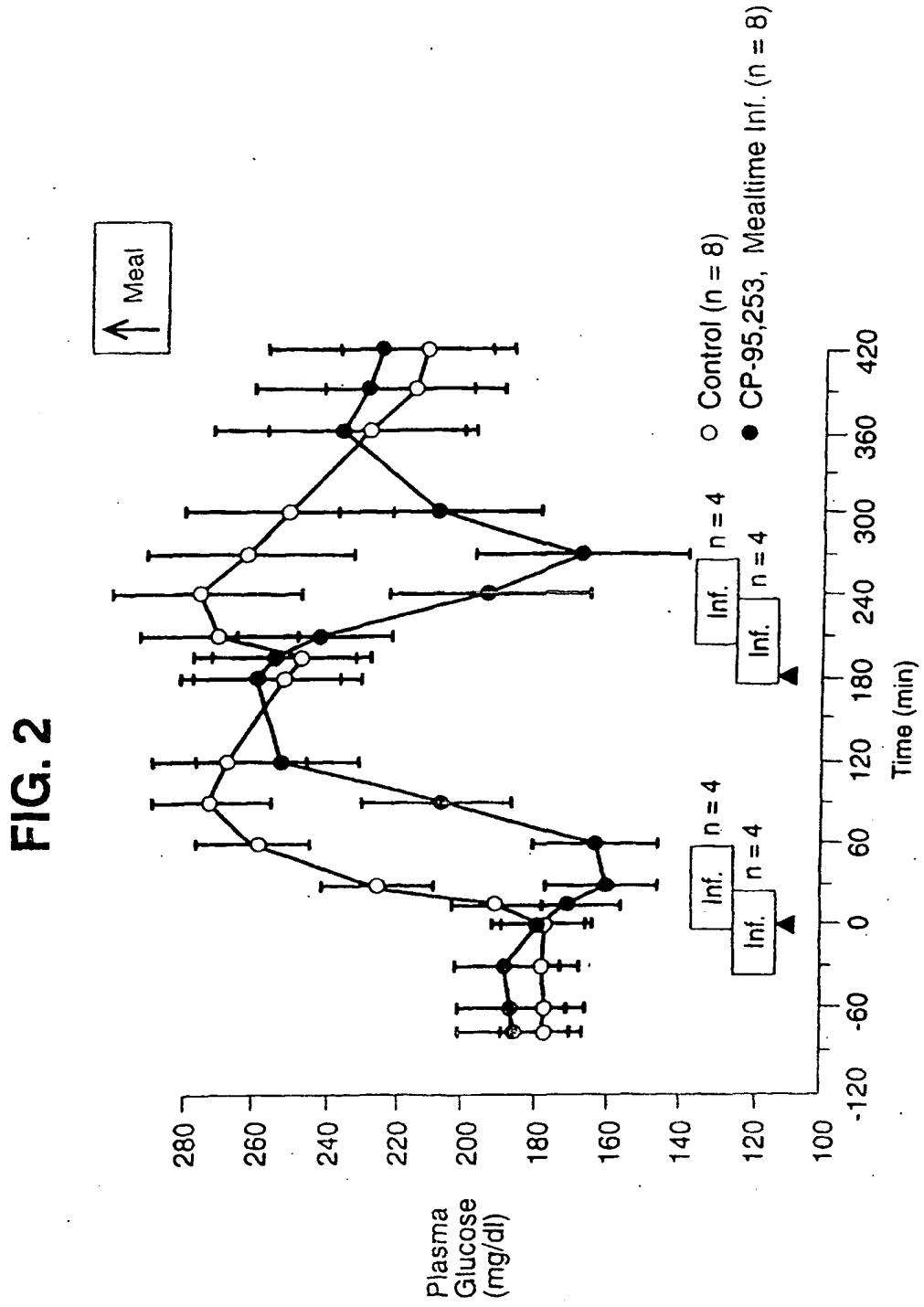

Meal
Control (n = 8)
CP-95,253, Mealtime Inf. (n = 8)
Inf.
n = 4
Inf.
n = 4
Inf.
n = 4
Inf.
n = 4
Plasma Glucose (mg/dl)
280
260
240
220
200
180
160
140
120
100
-120
-60
0
60
120
180
240
300
360
420
Time (min)

FIG. 3

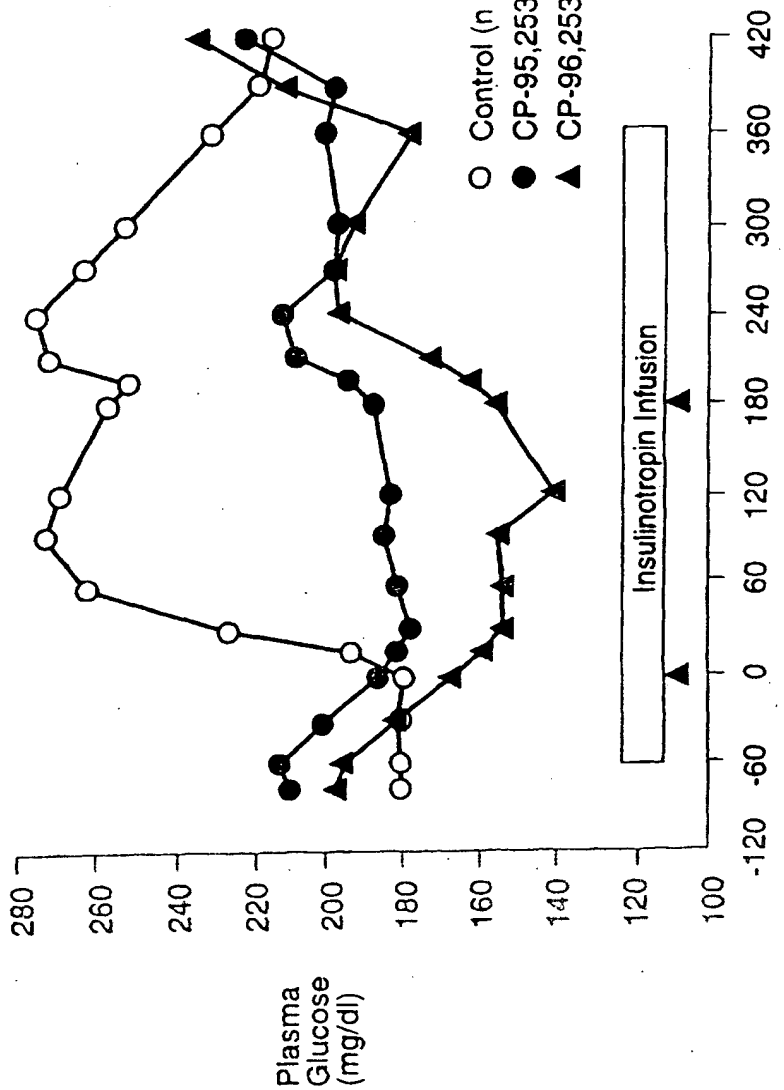

Plasma Glucose (mg/dl)
280
260
240
220
200
180
160
140
120
100
-120
-60
0
60
120
180
240
300
360
420
Insulinotropin Infusion
Control (n = 8)
CP-95,253, 2 ng/kg/min (n = 8)
CP-96,253, 4 ng/kg/min (n = 8)

FIG. 4

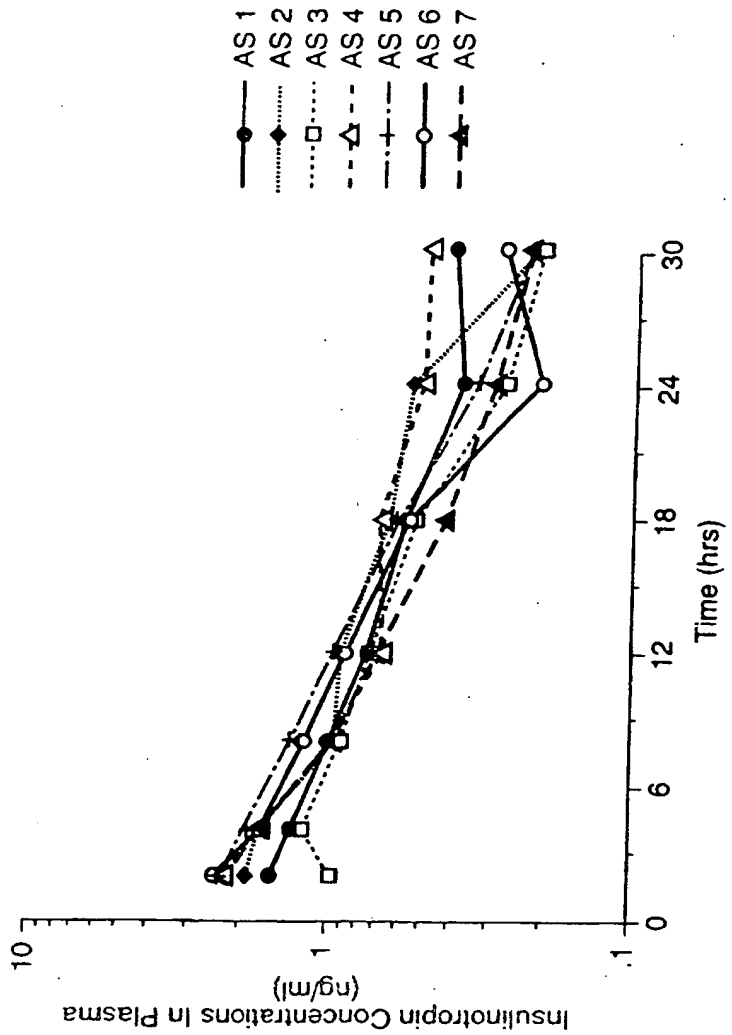

AS 1
AS 2
AS 3
AS 4
AS 5
AS 6
AS 7
Insulinotropin Concentrations In Plasma (ng/ml)
10
1
.1
0
6
12
18
24
30
Time (hrs)

FIG. 5

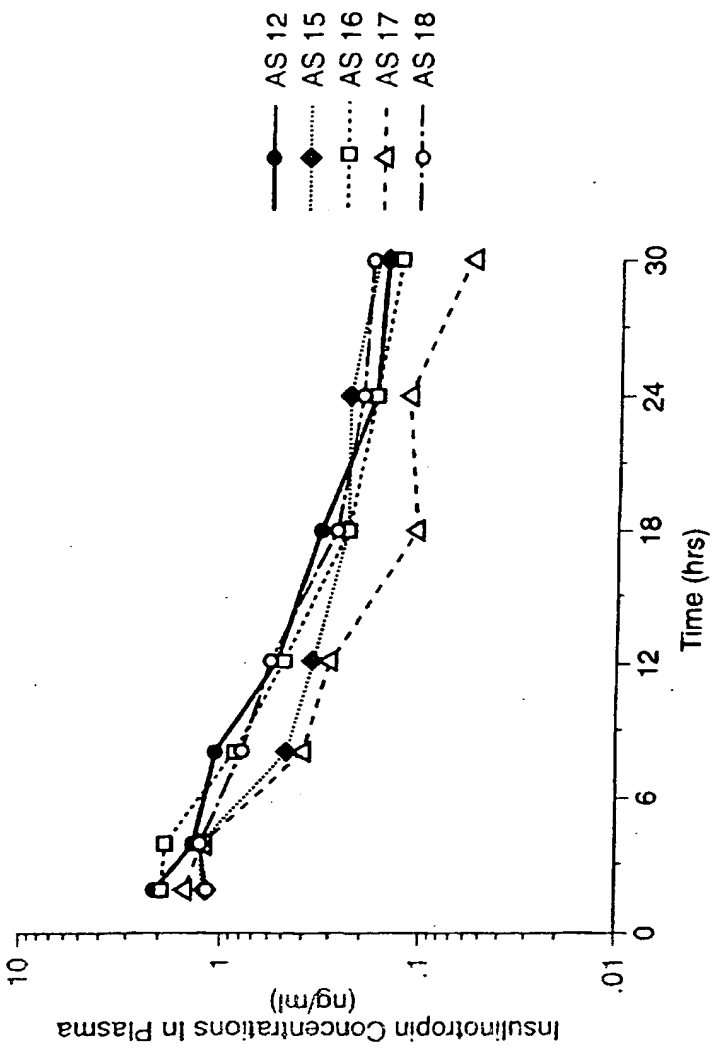

AS 12
AS 15
AS 16
AS 17
AS 18
Insulinotropin Concentrations In Plasma (ng/ml)
10
1
.1
.01
0
6
12
18
24
30
Time (hrs)

FIG. 6

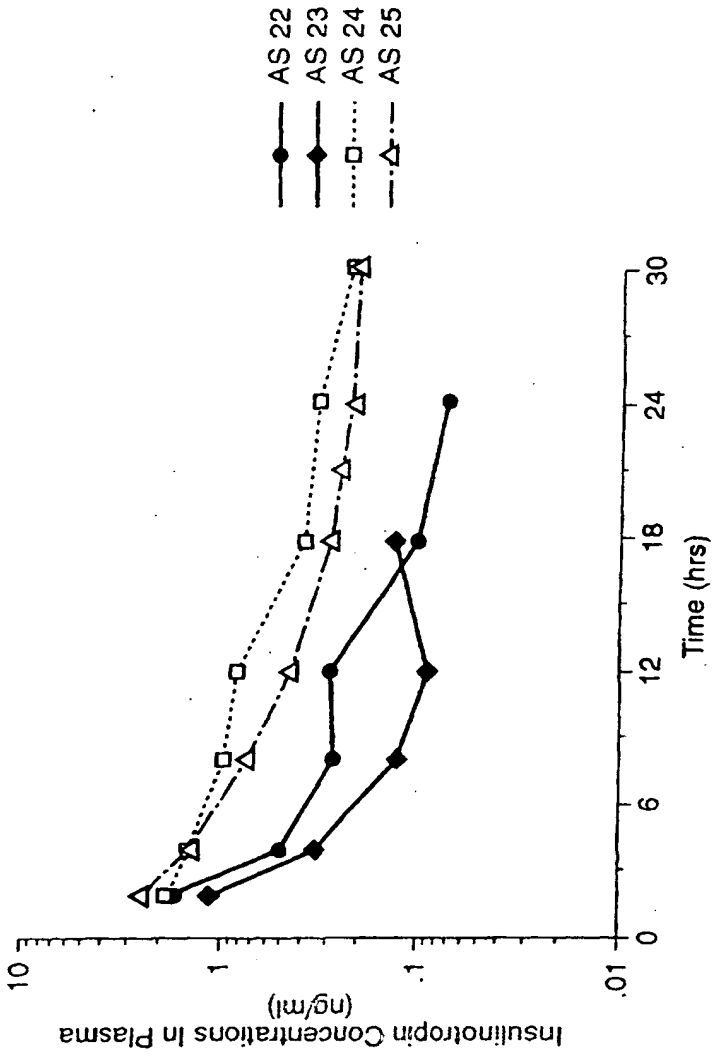

AS 22
AS 23
AS 24
AS 25
Insulinotropin Concentrations In Plasma
(ng/ml)
10
1
.1
.01
0
6
12
18
24
30
Time (hrs)

FIG. 7

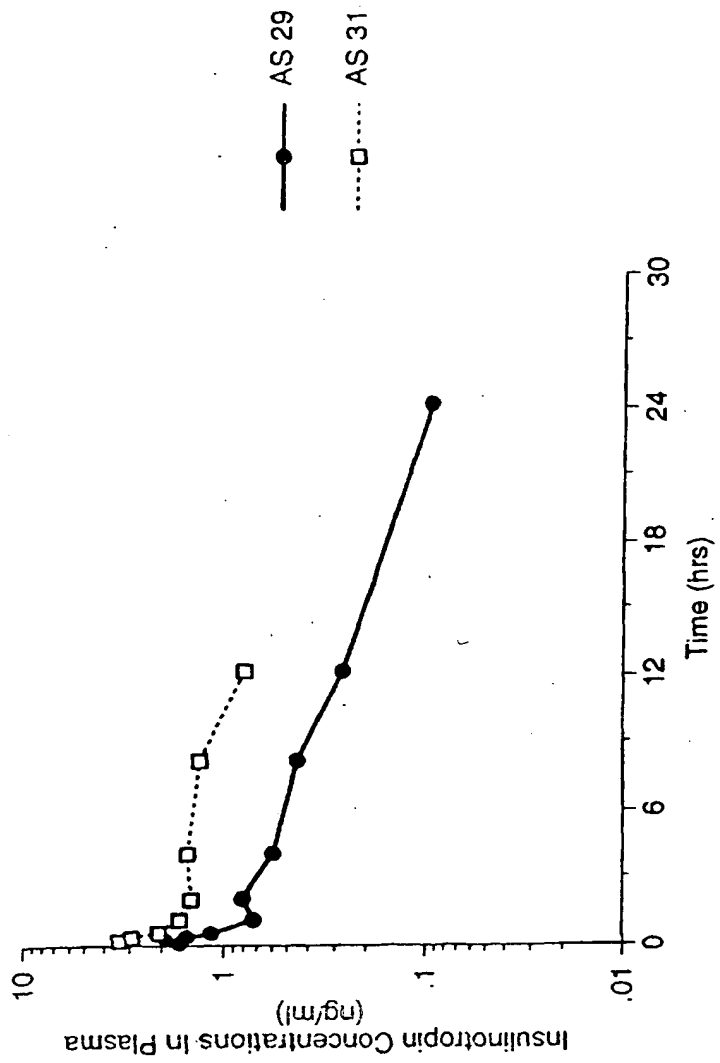

AS 29
AS 31
Insulinotropin Concentrations in Plasma (ng/ml)
10
1
.1
.01
0
6
12
18
24
30
Time (hrs)

FIG. 8

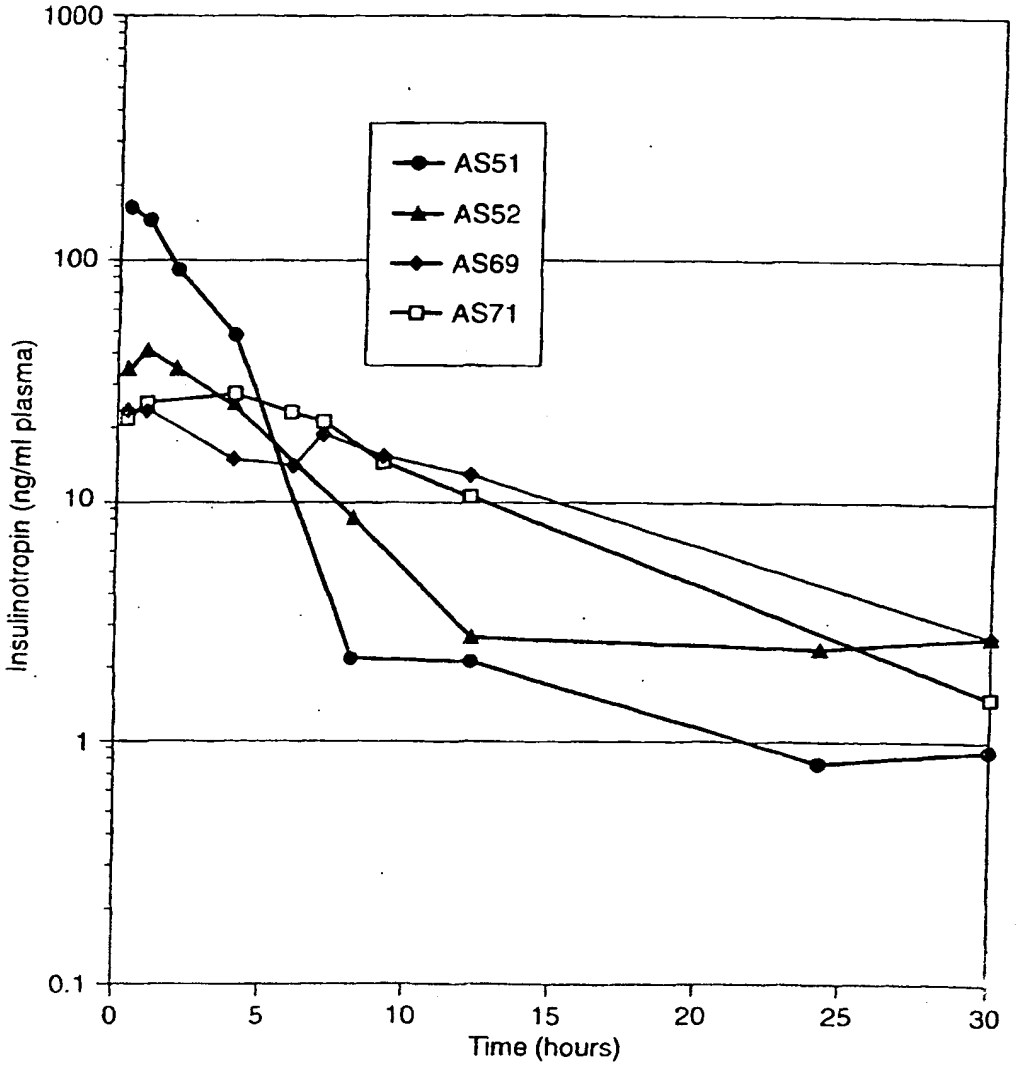

1000
100
10
1
0.1
Insulinotropin (ng/ml plasma)
0
5
10
15
20
25
30
Time (hours)
AS51
AS52
AS69
AS71

FIG. 9

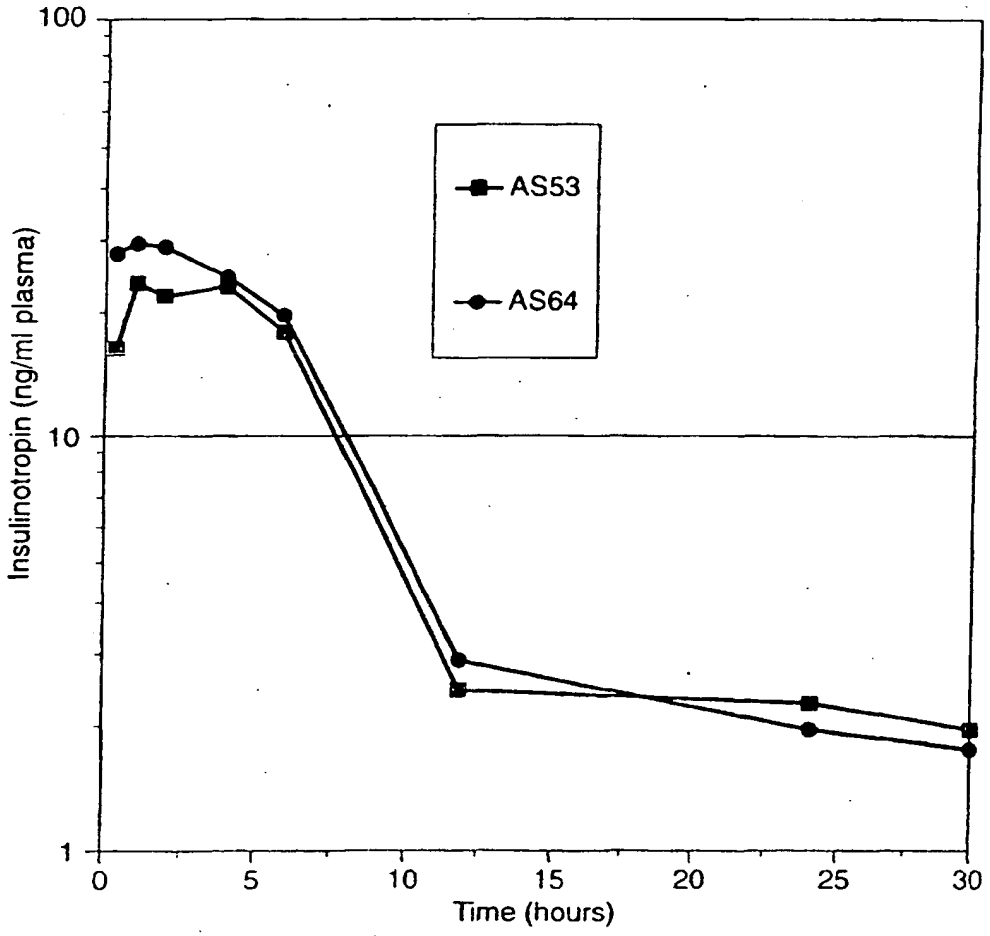
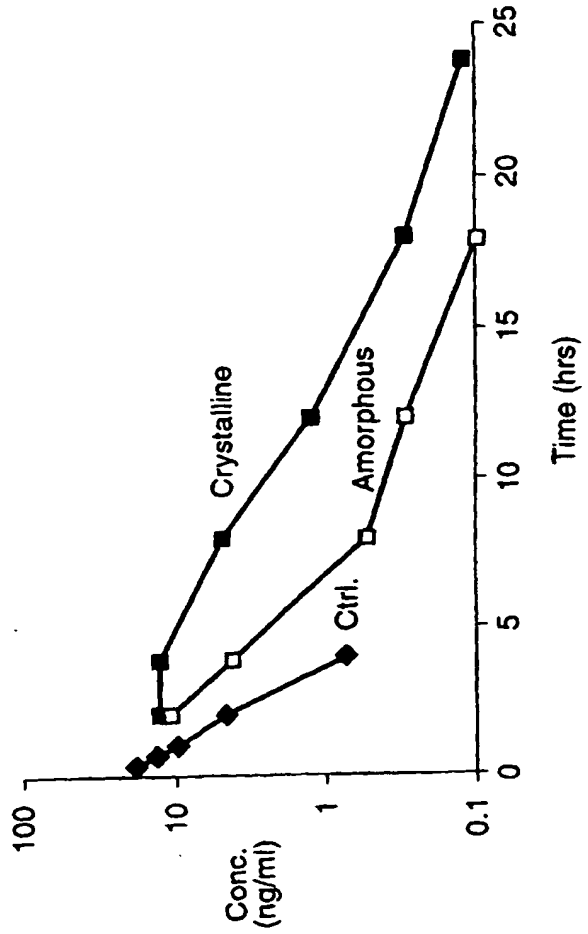

100
10
1
Insulinotropin (ng/ml plasma)
AS53
AS64
0
5
10
15
20
25
30
Time (hours)

FIG. 10


Crystalline
Amorphous
Ctrl.
Conc. (ng/ml)
100
10
1
0.1
0
5
10
15
20
25
Time (hrs)

Guidance for Industry

Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact Wallace P. Adams (301) 594-5651 (CDER).

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
June 1999

Guidance for Industry

Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
June 1999

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May 27, 1999

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Draft - Not for Implementation

GUIDANCE FOR INDUSTRY[1]

Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action

I. INTRODUCTION

This guidance is intended to provide recommendations to applicants who are planning product quality studies to measure bioavailability (BA) and/or establish (BE) in support of new drug applications (NDAs) or abbreviated new drug applications (ANDAs) for locally acting drugs in nasal aerosols (metered-dose inhalers (MDIs)) and nasal sprays (metered-dose spray pumps). Product quality includes chemistry, manufacturing, and controls (CMC), microbiology, certain BA information, and BE information (i.e., information that pertains to the identity, strength, quality, purity, and potency of a drug product). Product quality BA and BE are reflective of potency, in that release of the drug substance from the drug product should be assessed and controlled to achieve a reproducibly potent product. BA studies can address many questions, but this guidance discusses studies that focus on product performance (i.e., release of drug substance from drug product). A BE study is normally used to compare a test product (T) to a precursor product (R) — the to-be-marketed product is compared to a pivotal clinical trial material; a generic product is compared to a reference listed drug .

Product quality approaches should be similar for all nasal aerosols and nasal sprays where the active ingredient/active moiety is intended for local action, regardless of drug or drug class. This guidance should be used with other, more general CMC and BA and BE guidances available from CDER (Internet, http://www.fda.gov/cder/guidance/index.htm). Product quality information is different from, yet complementary to, the clinical safety and efficacy information that supports approval of an NDA. For information about the type of safety and efficacy information that may be needed for a new active ingredient/active moiety intended for local action in the nose, or for a new product such as a nasal aerosol that may include an active ingredient/active moiety previously approved in a nasal spray, appropriate CDER review staff should be consulted.

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[1] This guidance has been prepared by the Oral Inhalation and Nasal Drug Products Technical Committee, Locally Acting Drug Products Steering Committee, Biopharmaceutics Coordinating Committee, with contributions from the Inhalation Drug Products Working Group, the Chemistry, Manufacturing, and Controls Coordinating Committee, in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration. This guidance represents the Agency's current thinking on product quality information related to inhalation aerosols and metered dose spray pumps for nasal delivery. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

This guidance covers BA and BE studies of prescription corticosteroids, antihistamines, anticholinergic drug products, and the over-the-counter (OTC) mast-cell stabilizer cromolyn sodium. The guidance does not cover studies of nasal sprays included in an applicable OTC monograph or studies of (1) metered-dose products intended to deliver drug systemically via the nasal route[2] or (2) drugs in nasal nonmetered dose atomizer (squeeze) bottles that require premarket approval.

Note: Detailed chemistry, manufacturing, and controls information relevant to nasal aerosols and nasals sprays are presented in two draft guidances, *Metered Dose Inhaler (MDI) and Dry Powder Inhaler(DPI) Drug Products — Chemistry, Manufacturing, and Controls Documentation* (October 1998) and *Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Product* (available June 1999). These draft guidances, when finalized, will provide complementary information on the BA/BE testing methods recommended in this guidance.

II. BACKGROUND

A. BIOAVAILABILITY AND BIOEQUIVALENCE DATA

Bioavailability is defined at 21 CFR 320.1 as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action." *Bioequivalence* is defined as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study." BA and BE are closely related, and the same approach to measure BA in an NDA should generally be followed in establishing BE for an NDA or ANDA. Although BA may be comparative, establishing BE specifically involves a comparison of the BA of one product with the BA of another product. BE is usually established using (1) criteria based on means and/or variances for BA measures, (2) BE intervals (goalposts), which are standards to allow a determination of equivalence when confidence intervals are computed using the specified criteria, and (3) confidence intervals for the criteria.

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[2] 21 CFR 341. Cold, Cough, Allergy, Bronchodilator, and Antiasthmatic Drug Products for Over-the-Counter Human Use.

BA and BE data should be provided in accordance with the regulations.[3] BA and BE may be established by in vivo (pharmacokinetic (PK), pharmacodynamic (PD), or clinical) and in vitro studies, or, with suitable justification, by in vitro studies alone.[4] BA and BE assessments for locally acting nasal aerosols and sprays are complicated because delivery to the sites of action does not occur primarily after systemic absorption. Droplets and/or drug particles are deposited topically, then absorbed and becomes available at local sites of action. Systemic exposure following nasal administration can occur either from drug absorbed into the systemic circulation from the nasal mucosa, or after ingestion and absorption from the gastrointestinal tract. A drug administered nasally and intended for local action is therefore likely to produce systemic activity, although plasma levels of the drug do not reflect the amount of the drug reaching nasal sites of action. For these reasons, BA and BE studies should consider both local delivery and systemic exposure or systemic absorption.

1. Local Delivery BA/BE Concepts

For local delivery, BA is determined by several factors, including release of drug substance from the drug product and availability to local sites of action. Release of drug from the drug product is characterized by distribution patterns and droplet or drug particle size within the nose that are dependent upon drug substance, formulation, and device characteristics. Availability to local sites of action is a function of the above release factors, as well as drug dissolution in the case of suspension products, absorption across mucosal barriers to nasal receptors, and rate of removal from the nose. From a product quality perspective, the critical issues are release of drug substance from drug product and delivery to the mucosa. Other factors are of lesser importance. A critical question in assessing product quality BA and BE is the extent to which one can rely on in vitro methods alone, or upon in vitro methods plus clinical endpoints, to measure (benchmark) BA and/or establish BE. In vitro methods are less variable, easier to control, and more likely to detect differences between products if they exist, but the clinical relevance of these tests, or the magnitude of the differences in the tests, is not always clearly established. Clinical endpoints may be highly variable and relatively insensitive in detecting differences between products, but can unequivocally establish effectiveness.

In this guidance, the recommended approach for solution formulations of locally acting nasal drug products is to rely on in vitro methods to assess BA and BE. This approach is based on the assumption that in vitro studies would be more sensitive indicators of drug delivery to nasal sites of action than would be clinical studies. Drug particle size

[3] 21 CFR 320.21, Requirements for submission of in vivo bioavailability and bioequivalence data.

[4] 21 CFR 320.24, Types of evidence to establish bioavailability or bioequivalence.

distribution (PSD) in suspension formulations has the potential to influence the rate and extent of availability to nasal sites of action and to the systemic circulation. For suspension formulation products, however, due to the inability to adequately characterize drug PSD (see section V.B.2), in vivo studies should be conducted as part of the studies establishing product quality BA and BE. In vitro studies should be coupled with a clinical study for BA, or a BE study with a clinical endpoint for BE, to determine the delivery of drug substance to local nasal sites of action. An in vivo systemic exposure or systemic absorption study should also be conducted for suspensions (see section II.A.2). For solution formulations, see section IV.B.1.

2. Systemic Exposure and Systemic Absorption BA/BE Concepts

Locally acting drugs are intended to produce their effects upon delivery to nasal sites of action without relying upon systemic absorption. Although systemic absorption may contribute to clinical efficacy for certain corticosteroids and antihistamines, the consequences of systemic absorption (e.g., HPA suppression by corticosteroids) are generally undesirable. In the absence of validated in vitro methodology for characterization of drug PSD for suspension products, and when measurable plasma levels can be obtained, this guidance recommends PK studies to measure systemic exposure BA or establish systemic exposure BE (section VII). For suspension products that do not produce sufficient concentrations to assess systemic exposure, clinical studies or BE studies with a clinical endpoint should be used to measure systemic absorption BA and establish systemic absorption BE, respectively (section VIII). For a schematic representation of recommended studies, see the Decision Tree for In Vivo Product Quality BA and BE Studies for Nasal Aerosols and Nasal Sprays (p. 35).

BA recommendations in this guidance are limited to *product quality* BA. For investigational new drugs (INDs) and NDAs, not only should product quality BA be provided, but BA/PK studies should also be included in the Human Pharmacokinetics section (Item 6) of the NDA for nasal aerosols and nasal sprays for local action, whether formulated as solutions or suspensions, and whether or not validated methods of determining drug PSD are available. These PK data provide biopharmaceutic and clinical pharmacology information beyond product quality BA characterization.

B. CMC TESTS AND IN VITRO BA TESTS (NONCOMPARATIVE) VERSUS BE TESTS (COMPARATIVE)

Generally CMC tests help characterize the identity, strength, quality, purity, and potency of the drug product and assist in setting specifications (tests, methods, acceptance criteria) to allow batch release. These tests have a different purpose than do BA/BE tests, which focus on release

of drug substance from drug product. Some of the in vitro BA/BE tests described in this guidance for nasal aerosols and sprays may be the same as CMC tests for characterization and/or batch release. A specification (test, method, acceptance criterion) for a CMC test for batch release is usually based on general or specific manufacturing experience. For example, a CMC test such as dose content uniformity has acceptance criteria based on repeated manufacturing of batches. Bioequivalence limits for BE studies are not usually based on manufacturing experience, but are part of equivalence comparisons between test and reference products. Equivalence comparisons normally include (1) a criterion to allow the comparison, (2) a confidence interval for the criterion, and (3) a BE limit for the criterion. BE limits may be based on a priori judgments and may be scaled to variability of the reference product (see Section IX). When conducted premarket for an NDA, some of the in vitro BA tests described in this guidance can be noncomparative and serve primarily to document (benchmark) the product quality BA of a pioneer product.

III. FORMULATION AND CONTAINER AND CLOSURE SYSTEM

A. FORMULATION

Particle size, morphic form, and state of solvation of the active ingredient have the potential to affect the BA of the drug product as a result of different solubilities and/or rates of dissolution. For an ANDA of a suspension formulation, the PSD of the active drug in the dosage form should be the same as that of the reference listed drug, as discussed in Section V.B. Comparative information on the morphic form of the drug particles, and size and number of drug aggregates in the dosage form, should be provided. In addition, documentation of the same anhydrous or solvate form should be provided. For suspension formulations marketed in more than one strength, the drug substance in each strength product should be micronized under identical parameters, and the PSD of the resultant bulk drug should be identical in each strength product.

B. CONTAINER AND CLOSURE SYSTEM

Nasal aerosols consist of the formulation, container, valve, actuator, dust cap, associated accessories (e.g., spacers), and protective packaging, which together constitute the drug product. Similarly, nasal sprays consist of the formulation, container, pump, actuator, protection cap, and protective packaging, which together constitute the drug product.

For nasal aerosols and nasal sprays approved under an ANDA, BE should be documented on the basis of validated in vivo and vitro tests, or, in some cases, validated in vitro tests alone may be appropriate. Assurance of equivalence on the basis of in vitro tests is greatest when the test product uses the same brand and model of devices (particularly the metering valve or pump and the actuator) as used in the reference product. If this is not feasible, valve, pump, and actuator

designs should be as close as possible in all critical dimensions to those of the reference product. Metering chamber volumes should be the same. For nasal aerosols, overall actuator design (Byron 1990), including actuator orifice diameter, should be the same. For a nasal spray, spray characteristics may be affected by features of the pump design, including the precompression mechanism, actuator design, including specific geometry of the orifice (Kublic and Vidgren 1998), and design of the swirl chamber. The external dimensions of the test actuator should ensure comparable depth of nasal insertion to the reference actuator. A test product should attain prime within the labeled number of actuations for the reference product. Consideration should be given to the *dead volume* of the device, including the internal diameter and length of the diptube, because this volume can influence the number of actuations required to prime a spray pump.

IV. DOCUMENTATION OF BIOAVAILABILITY AND BIOEQUIVALENCE

A. INDs/NDAs

For INDs/NDAs, in vitro BA studies for solutions and suspensions, and in vivo studies for suspensions, should be provided. These data are useful as a benchmark to characterize the in vitro performance, and for suspensions, the in vivo performance of the product based on the clinical efficacy and either systemic exposure for a PK study, or systemic absorption for a clinical safety study. Where the formulation and/or method of manufacture of the pivotal clinical trial product changes in terms of physicochemical characteristics of the drug substance, the excipients, or the device characteristics, BE data using in vitro tests (for solutions and suspensions) and in vivo tests (for suspensions) may be useful in certain circumstances during the preapproval period to ensure that the to-be-marketed product (T) is comparable to very similar clinical trial batches and/or to batches used for stability testing (R) (section V.A.1). Sponsors should discuss the usefulness of these BE approaches with appropriate CDER review staff.

B. ANDAs

1. Solution Formulations

In vivo studies, such as seasonal allergic rhinitis (SAR) studies to establish equivalent delivery to nasal sites, or HPA suppression studies for corticosteroids to establish equivalent systemic absorption, are not considered necessary for nasally administered solution drug products intended for local action. Thus, reliance on in vitro tests alone to document BE is suitable for nasal solution formulation products intended for local action. This approach is based on an understanding that for solution products, equivalent in vitro performance, inactive ingredients that are qualitatively (Q_1) the same and quantitatively (Q_2) essentially the same as the inactive ingredients in the reference listed drug, and adherence to container and closure recommendations of section III will ensure comparable

delivery to the nasal mucosa and to the gastrointestinal tract. Quantitatively *essentially the same* has been determined by CDER to mean that the concentration or amount of the inactive ingredient(s) in the test product should not differ by more than ±5 percent of the concentration or amount in the reference listed drug. Suggested methodology and validation approaches for the recommended tests are provided in section V. Suggested methods to allow comparisons using a criterion, BE limits, and a confidence interval approach are discussed in section IX. When in vitro data fail to meet acceptance criteria, the applicant is encouraged to modify the test product to attain equivalent in vitro performance. Because of insensitivity to potential differences between T and R, in vivo studies will not be sufficient in the face of in vitro studies that fail to document BE.

2. Suspension Formulations with PK Systemic Exposure Data

To document BE for suspension nasal formulation products intended for local action, both in vitro and in vivo data should be used. Inactive ingredients also should be qualitatively (Q_1) the same and quantitatively (Q_2) essentially the same as the inactive ingredients in the reference listed drug, and the container and closure recommendations of section III should be followed. In vivo studies should include both a pharmacokinetic study (systemic exposure) and a BE study with a clinical endpoint (local delivery). This approach is only applicable for those suspension formulation products that produce sufficiently high drug concentrations in blood or plasma after nasal administration to obtain meaningful AUC and C_{max} data. Methodology and validation approaches for the recommended tests are provided for the in vitro studies in section V, and for the in vivo studies in sections VI and VII. As with solutions, in vivo studies will not be sufficient in the face of in vitro studies that fail to establish BE (i.e., in vitro BE studies that fail to meet the statistical test discussed in section IX result in a failed BE study) even though the BE study with a clinical endpoint or the PK study meets the statistical test.

3. Suspension Formulations without PK Systemic Exposure Data

For suspension nasal formulation products, inactive ingredients should be qualitatively (Q_1) the same and quantitatively (Q_2) essentially the same as the inactive ingredients in the reference listed drug, and the container and closure recommendations of section III should be followed. In addition, for those products intended for local action that produce blood or plasma levels that are too low for adequate measurement, given current assay constraints, a BE study with a clinical endpoint to establish equivalent local delivery to nasal sites (section VI) and a study with a pharmacodynamic or clinical endpoint to establish equivalent systemic absorption (section VIII) are recommended. In vivo studies that meet the statistical test will not be sufficient in the face of in vitro studies that fail to document BE.

C. POSTAPPROVAL CHANGE

For an NDA submitted under 505(b)(1) of the Food, Drug, and Cosmetic Act, the primary need for BE documentation would be between the reference product before and the reference product after very limited changes. For an ANDA and for an NDA submitted in accordance with section 505(b)(2) of the Food, Drug, and Cosmetic Act, the primary documentation of BE for the changed product is the reference or pioneer product. At this time, no guidance is available as to when BE should be redocumented in the presence of any postapproval changes, either for an NDA or ANDA. Sponsors planning such changes should contact the appropriate review division prior to instituting the change.

V. BIOAVAILABILITY AND BIOEQUIVALENCE: IN VITRO STUDIES

A. BATCHES AND DRUG PRODUCT SAMPLE COLLECTION

1. INDs/NDAs

In vitro product quality BA studies for nasal aerosols and sprays should generally be performed on samples from three batches. The batches should include a pivotal clinical trial batch, a primary stability batch, and if feasible, a production scale batch, to provide linkage of in vitro performance to in vivo data. If a production scale batch is not available, a second pivotal clinical trial batch can be substituted.

The above BA batches should be equivalent to the to-be-marketed product. The manufacturing process of these batches should simulate that of large-scale production batches for marketing (additional information on large-scale batches is provided in the International Conference on Harmonisation (ICH) guidance for industry Q1A *Stability Testing of New Drug Substances and Products* (September 1994), section V.B). Complete batch records, including batch numbers of device components used in the batches, should accompany the BA submission.

In vitro BA studies are intended to characterize the means and variances of measures of interest for canisters (nasal aerosols) or bottles (nasal sprays) within a batch and between batches, where applicable. However, under 21 CFR 320.1 and 320.21, the studies may be noncomparative to other formulations or products. The in vitro tests and metrics are described in section V.B. The test method or standard operating procedure (SOP) for each test should accompany the data in the submission. The recommended number of canisters or bottles of each batch to be used in the above studies, and recommendations for statistical analyses, are described in section IX.

2. ANDAs

In vitro BE studies for nasal aerosols and sprays should generally be performed on samples from each of three batches of the test product and three batches of the reference listed drug. Test product samples should be from the primary stability batches used to establish the expiration dating period. Test product should preferably be manufactured from three different batches of the drug substance, different batches of critical excipients, and container and closure components. For nasal sprays formulated as solutions, in vitro BE tests can alternatively be performed on three sublots of product prepared from one batch of the solution.[5]

The above BE batches should be equivalent to the to-be-marketed product. The manufacturing process of these batches should simulate that of large-scale production batches for marketing (ICH Q1A *Stability Testing of New Drug Substances and Products* (September 1994), section V.B). Complete batch records, including batch numbers of device components used in the batches or sublots (for solution nasal sprays) should accompany the BE submission.

Reference product samples should be from three different batches available in the marketplace. The recommended in vitro tests and metrics are described in section V.B. The recommended number of canisters or bottles of each product and batch to be used in the above studies, and recommended statistical approaches, including suggested boundaries for each of the studies, are described in section IX.

B. TESTS AND METRICS

In vitro BA and BE for locally acting drugs delivered by nasal aerosol or nasal spray are characterized by six tests:

1. Dose or Spray Content Uniformity Through Container Life
2. Droplet and Drug Particle Size Distribution
3. Spray Pattern
4. Plume Geometry
5. Priming and Repriming
6. Tail Off Profile

---

[5] For solution formulation nasal sprays, variability in in vitro BE study data between batches is expected to be due primarily to variability in the device components of the product rather than in the solution. Therefore, a single batch of solution may be split-filled into three equal size sublots of product. The sublots should be prepared from three different batches of the same device (pump and actuator) components.

The in vitro test information described below is summarized in Table 1 (p. 35).

All in vitro tests should be conducted on test canisters or bottles selected in a randomized manner from the test batch, including units from the beginning, middle and end of the production run. BE tests should be conducted in a blinded manner, or should use another approach that removes potential analyst bias, without interfering with product performance. Automated actuation stations are recommended for all comparative in vitro BE tests to decrease variability in drug delivery due to operator factors (including removal of potential analyst bias in actuation) and increase the sensitivity for detecting potential differences between products in any of the above tests.[6] The blinding procedure should also be extended to postactuation evaluations. The randomization procedure and the test method or SOP for each test should accompany the data in the submission.

1. Dose or Spray Content Uniformity Through Container Life

Sampling apparatus for collection of dosage units from aerosols is described in *U.S. Pharmacopeia 23/National Formulary 18* (Tenth Suppl, 15 May 1998). A suitable apparatus should be used for collection of dosage units from nasal sprays. For both solution and suspension formulations of nasal aerosols and nasal sprays, the mass of drug delivered per single (unit) dose should be determined based on a stability-indicating chemical assay. A single dose represents the minimum number of sprays per nostril specified in the product labeling. For a nasal product for which the minimum single usual dose is one actuation in each nostril, the single dose should be based on one actuation. For a nasal product for which the minimum usual dose is two actuations in each nostril, the single dose should not exceed two actuations. For BA and BE studies, dose or spray content uniformity data should be determined on primed units at the beginning of unit life, at the middle of unit life, and at the end of unit life[7] for nasal aerosols, and at beginning and end of unit life for nasal sprays. Mean dose or spray content uniformity and variability

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[6] Automated actuation stations may be stand-alone systems or accessories for laser diffraction instruments. Stations may include settings for actuation force, actuation velocity, hold time, return time, delay time between actuations, length of stroke, and number of actuations. Selection of appropriate settings should be relevant to proper usage of the nasal aerosol or nasal spray by the trained patient, and should be documented based on exploratory studies in which actuation force, actuation time, and other relevant parameters are varied. These studies should accompany the validation data. Selected settings used for the comparative in vitro study should be specified in the SOP for each test for which the automatic device is employed.

[7] Based on the labeled number of full medication doses, this guidance uses the terms *beginning life stage, middle life stage*, and *end life stage* interchangeably with the terms *beginning of unit life* (the first actuation(s) following the labeled number of priming actuations); *middle of unit life* (the actuation(s) corresponding to 50 percent of the labeled number of full medication doses); and *end of unit life* (the actuation(s) corresponding to the label claim number of full medication doses).

in content uniformity is to be determined based on within and between canister or bottle data, and, for nasal aerosols and suspension formulation nasal sprays, between batch data. Analytical data should be validated,[8] and the analytical validation report should accompany the content uniformity report. For BE data, equivalence of T and R data should be based on the methodology of section IX.A.1.

2. Droplet and Drug Particle Size Distribution (PSD)

To increase nasal deposition and minimize deposition in the lungs and GI tract, aerosol droplets should generally have a mass median aerodynamic diameter (MMAD) greater than 10 to 20 microns (Task Group on Lung Dynamics, 1966). As MMAD decreases over the 5-20 micron range, the Task Group report indicates that reduced nasopharyngeal deposition and increased pulmonary deposition occur. Droplet size distribution measurements are thus critical to delivery of drug to the nose. For BA and BE, studies of droplet size distribution and PSD by validated methods should be performed. For suspension products, drug particle size may be important to rate of dissolution and availability to sites of action within the nose. Therefore, drug or drug and aggregate PSD should be characterized in the formulation both within the can or bottle and within the aerosolized droplets. Present agency experience suggests that drug and drug aggregate PSD characterization cannot be acceptably validated for nasal aerosols and nasal sprays. In this circumstance, drug and drug aggregate PSD studies should be performed, and these supportive characterization data, along with available validation information, should be submitted.

a. Particle size distributions

Droplet Size Distribution

For all nasal aerosols and nasal sprays, whether formulated as solution or suspension products, droplet size distribution should be determined utilizing a method suitable for fully characterizing the droplet size. Laser diffraction methodology, or appropriately validated alternate methodology, is recommended.

Particle Size Distribution

For all nasal aerosols and nasal sprays, whether formulated as solution or suspension products, PSD should be determined using a suitable aerodynamic

[8] A draft guidance for industry is under development on analytical procedures, validation data, and samples for drug substances and drug products.

method (e.g., multistage cascade impactor (CI), multistage liquid impinger (MSLI)).

Drug and Aggregate PSDs

Nasal spray suspension formulations typically contain micronized drug within an aqueous vehicle with partially undissolved suspending agents and other ingredients. Nasal aerosol suspension formulations contain micronized drug suspended within propellants, and may contain a surfactant and/or cosolvent. Light microscopy may be considered for estimating drug and drug aggregate PSD of these products.

b. Instrumental methods

Laser Diffraction

Laser diffraction is a nonaerodynamic optical method of droplet or particle sizing which measures the geometric size of droplets or particles in flight. To characterize the beginning, middle, and end of the plume, measurements should be made at three distances from the delivery orifice. Multiple actuations may be performed at each lifestage to assess precision. The droplet size distributions due to each actuation, and the means, standard deviations (SDs), and percent coefficients variation (CVs) should be reported. At each distance, measurements should be made at different delay times in order to characterize the size distribution of droplets or particles within the plume upon formation, as the plume has started to dissipate, and at some intermediate time (Sciarra and Cutie, 1989). Selected delay times may be based on obscuration levels or other suitable means.[9]

Droplet size distribution data (D_{10}, D_{50}, D_{90}), and span ((D_{90} - D_{10})/D_{50}) should be reported based on volume (mass). Droplet size distribution data by count (number of droplets) are not requested. All instrument/computer printouts should be submitted, including cumulative percent undersize tables, histograms of PSD, obscuration values, and other details and statistics. The manufacturer's recommended obscuration ranges for the laser diffraction instrument should be submitted.

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[9] *Obscuration* refers to the percentage of laser light obscured or scattered out of the beam by the sample, and is influenced by sample concentration and width of the plume. Following actuation, obscuration levels are initially low, increase as the plume develops, then decrease as the plume dissipates.

Comparative laser diffraction data are requested at beginning, middle, and end of unit life. For BE, statistical comparisons should be based on D_{50} and span.

Multistage Cascade Impaction (CI) or Multistage Liquid Impinger (MSLI)

Sizing of droplets or particles by CI or MSLI measures aerodynamic diameter based on inertial impaction, an important factor in the deposition of drug in the nasal passages. CI or MSLI data should be provided for all nasal sprays and nasal aerosols to characterize the size distribution of drug based on aerodynamic mass diameters. The greatest percentage of the emitted dose is deposited prior to or on the first stage of the CI for both nasal aerosols and nasal sprays. Thus, equivalence of aerodynamic drug particle size distribution of test and reference products, although conducted by validated procedures, does not ensure equivalent PSD of drug within the aerosolized droplets. Characterization of drug PSD by CI or MSLI, along with the other recommended in vitro tests, does not allow waiver of in vivo BE studies for suspension formulation products (see section II.A).

For BA and BE, CI or MSLI drug deposition profile data should be based on three size range groups. Group 1 includes summation of drug deposition in or on the valve stem, actuator, inlet port, and upper stage, which should have a nominal effective cutoff diameter (ECD) (e.g., greater than or equal to 9.0, 10.0, 13.0, or 16.0 microns). Group 2 includes drug deposition on the stage immediately below the upper stage (e.g., greater than or equal to 5.0 microns). Group 3 includes summation of drug deposition below the Group 2 stage, including the filter. For Group 1 only, deposition should also be reported for each of the individual accessories and the upper stage. Deposition should be reported in mass units. Mass balance accountability (sum of all drug deposited from the valvestem to the filter) should be documented.

Selection of the most suitable cascade impactor may be influenced by the ECDs of stages of various brands of cascade impactors, the geometry of the induction port, and other factors. Studies should use the fewest number of actuations justified by the sensitivity of the analytical method (generally not exceeding 10), in order to be more reflective of the PSD of individual doses. Analytical data should be based on a validated chemical assay. The analytical validation report should accompany the CI data report. The SOP or validation report should indicate the minimum quantifiable amount of drug deposited on each of the three groups of deposition sites and on each accessory or stage of the Group 1 data.

For BA and BE, cascade impactor data are requested at the beginning and end of unit life. Middle of unit life data are not requested. For BE, statistical

comparisons of drug deposition on the three groups should be based on profile analysis (section IX.D).

Light Microscopy

Light microscopy may provide drug and aggregate PSD data. However, the method is limited in its ability to fully characterize PSD by the resolution limit of light microscopy (about 0.5 micron or higher) which may not be adequate for sizing micronized drug. A second limitation is potential difficulty in distinguishing drug from undissolved excipient in suspension formulation nasal sprays. Due to these limitations, acceptable validation of the microscopic data may not be possible. In the presence of these limitations, this guidance recommends that comparative drug and aggregate PSD data should be submitted as supportive BA and BE characterization data for suspension formulation nasal aerosols and sprays. The occurrence of drug particles and aggregates within appropriate size ranges should be tabulated for each analysis, and histograms of the drug and aggregate PSD should be provided. Count median diameter (CMD) and geometric standard deviation (GSD) based on single particle data (aggregates excluded) should be provided. Studies of nasal sprays should include test product placebo to provide an estimate of the occurrence of apparent drug particles (*false positives*) due to undissolved excipient. PSD by light microscopy provides supportive BE information.

3. Spray Pattern

Spray pattern characterizes the spray following impaction on an appropriate target (e.g., a thin-layer chromatography (TLC) plate). It provides information about the shape and density of the plume following actuation. Spray patterns should be determined on single actuations at three appropriate distances from the actuator to the target at the beginning and end of unit life. The visualization technique should preferably be specific for the drug substance. End of unit life testing is requested to ensure comparability to performance at beginning of unit life. Clear, legible photographs or photocopies of the spray patterns, not hand-drawn representations obtained by tracing the pattern, should be provided. The widest (D_{max}) and shortest (D_{min}) diameters, and the ovality ratio (D_{max}/D_{min}) should be provided for each spray pattern. The SOP should include a figure describing the procedure for measurement of D_{max} and D_{min}. For BE, statistical comparisons should be based on ovality ratio and either D_{max} or D_{min} data (section IX.B).

Spray pattern and plume geometry (below) are recommended to assist in establishing functional equivalence of products as a result of differences in the device components of T and R products. Comparable spray pattern and plume geometry data for T and R,

combined with other in vitro tests (and in vivo studies for suspensions), ensure equivalent drug deposition patterns, resulting in equivalent delivery of drug to nasal sites of action and equivalent systemic exposure or absorption.

4. Plume Geometry

Plume geometry describes two side views, at 90 degrees to each other (two perpendicular planes) and relative to the axis of the plume, of the aerosol cloud when actuated into space. Plume geometry should be based on high-speed photography or other suitable methods. Photographs should be of high quality and should clearly show the dense cloud and individual large droplets or agglomerates of droplets in the vicinity of the cloud. Plume geometry may be performed only at the beginning of unit life. Plumes should be characterized at three or more times after a single actuation, chosen to characterize the plume early upon formation, as the plume has started to dissipate, and at some intermediate time. Photographs of plumes should be used to measure plume length, plume width, and plume (spray cone) angle. All photographs and data characterizing the plume dimensions in two planes should be submitted, including the scale used to indicate actual size. Comparative BE data are supportive (section IX.C).

5. Priming and Repriming

Priming and repriming data provide information to ensure delivery of the labeled dose of drug, and thus are part of the in vitro BA and BE assessment. Similar studies should be conducted on nasal sprays. For products approved under an NDA, priming and repriming data based on single actuations should be provided for multiple orientations.

For products approved under an ANDA, the labeling is the same as that for the reference listed drug, except for specific changes described in the regulations (21 CFR 314.94(a)(8)(iv). For nasal sprays and some nasal aerosols, the reference product labeling (package insert and/or patient package insert) describes the number of actuations necessary to prime the product on initial use and on repriming following one or more periods of nonuse (e.g., 24 hours and 7 days following last dose). Comparative priming and repriming data are requested to document that priming of the test product is attained within the number of priming actuations stated in the reference product labeling. For reference product nasal aerosols lacking priming recommendations, priming studies are recommended to characterize the test product relative to the reference product. In the absence of reference product priming recommendations, an adequate number of single actuations should be studied to ensure that test and reference products have each attained an emitted dose equal to the labeling claim. Repriming studies of test products are requested only when the reference product labeling includes repriming instructions.

Priming and repriming data for the test product in multiple orientations should be provided in the CMC portion of the ANDA submission. Therefore, comparative BE studies may be based on products stored in the valve upright position. For any nasal aerosol product in which the reference product labeling recommends storage in the valve down position, additional comparative priming and repriming data should be provided for this orientation. For suspension products, the unprimed canister or bottle should be shaken for a standardized time (e.g., 5 seconds) and a dose should then be immediately collected. For nasal aerosols, a standardized period (e.g., 30-60 seconds) should be allowed between successive actuations. Doses may be collected in the same apparatus used for the dose or spray content uniformity through container life test. When priming and/or repriming information is included in the labeling, comparison of equivalence should be based on the emitted dose of the single actuation immediately following the specified number of priming or repriming actuations (section IX.B). The emitted dose of each earlier actuation should also be provided. When priming information is not specified, the emitted dose of each successive actuation up to and including attainment of label claim should be provided. Comparative BE data in the absence of priming are supportive (section IX.C).

6. Tail Off Profile

Whereas dose or spray content uniformity conducted at the end of the labeled number of actuations ensures that the product delivers the labeled dose through the number of actuations stated in product labeling, the tail off profile characterizes the decrease in emitted dose following delivery of the labeled number of actuations (i.e., from end of unit life to product exhaustion). Tail off profile characteristics may vary as a function of valve or pump design, bottle geometry, and other factors, and may be characterized in terms of uniformity of decline, rate of decline, and intercanister or interbottle variability in unit dose (Schultz, 1995). For BA assessment, tail off data are noncomparative. For BE assessment, comparative tail off profiles are requested to ensure similarity in drug delivery as the product nears exhaustion. Data should be based on the emitted dose of individual actuations. Comparative BE data are supportive; however, the test product should be no more erratic in dose delivery than the reference product, and the rate of decline in delivery should be generally similar between products.

VI. BIOAVAILABILITY AND BIOEQUIVALENCE: CLINICAL STUDIES FOR LOCAL DELIVERY

A. GENERAL INFORMATION

The same adequate and well-controlled clinical trials in humans used to establish the safety and effectiveness of the drug product (21 CFR 314.126) may be used, in some cases, to establish BA

or, when comparative, BE (21 CFR 320.24). Although BA and BE studies with a clinical endpoint are sometimes incapable of showing a dose-response relationship and may not be consistently reproducible (21 CFR 320.24(b)(4)), they are sometimes the only means available to document BA and BE in drug products intended for local delivery and action. A number of FDA guidances provide information about the general conduct of clinical studies, including clinical studies to document BA and BE. These include: *General Considerations for Clinical Trials* (International Conference on Harmonisation (ICH) E8, December 1997); *Structure and Content of Clinical Study Reports* (ICH E3, July 1996); *Good Clinical Practice: Consolidated Guideline* (ICH E6, May 1997); and *Statistical Principles for Clinical Trials* (ICH E9, May 1997).

B. BE CLINICAL STUDY ENDPOINTS

Clinical evaluations should be made at baseline and during treatment. The efficacy endpoint should be patient self-rated total nasal symptom scores (TNSS). These most often include a composite score of runny nose, sneezing, nasal itching, and for drugs other than antihistamines, congestion. The efficacy endpoint should be expressed as change from baseline (pretreatment) of the TNSS, expressed in absolute units and percent change. In addition to the efficacy measures, all three study designs should incorporate safety assessments.

C. CLINICAL STUDY BATCHES

The product quality BA batch used for the study should be the same pivotal clinical trial batch used in the in vitro BA studies (section V.A). Where BE studies are needed for an NDA, the batches of test and reference products should be the same batches employed in the in vitro testing. The product quality batches used to establish the local delivery BE for an ANDA should be the test and reference batches employed in the in vitro BE testing.

D. CLINICAL BE STUDY DESIGNS AND SUBJECT INCLUSION CRITERIA

A BE study with a clinical endpoint to establish equivalent local delivery of drug from test and reference products to the nose should document sensitivity of the study to discriminate between differing doses (i.e., show a dose-response relationship). This documentation typically relies on the inclusion of a second dose of the reference product, and preferably of the test product, that may be higher or lower, to demonstrate that the efficacy response is different between the two doses. Doses may differ by two or fourfold, and to increase study sensitivity, the lower dose examined may be below the minimum labeled dose (e.g., one-half or one-quarter of the recommended dose, depending on the limitations of the formulation).

Although many clinical study design options may be considered to establish BE, outlined below are three suggested study designs for evaluating clinical responses for nasally administered drugs for seasonal allergic rhinitis (SAR): (1) traditional treatment, (2) day(s) in the park, and (3)

environmental exposure unit (EEU). The three study designs use SAR patients as the study population to document BE for all indications in product labeling for nasally administered drug products covered in this guidance. Recommended studies are designed as treatment studies rather than prophylaxis studies. Depending on the time to onset of therapeutic effect of the drug being tested, the medication effect can be evaluated after a single dose (e.g., antihistamines) or after short-term treatment (e.g., corticosteroids). In all three study designs, an assessment of onset of action and efficacy at the end of the dosing interval is recommended, because both measures are important clinically and may offer better dose discrimination.

Because specific study recommendations are not provided in this guidance, a protocol for a BE study with a clinical endpoint for a specific suspension drug product should be submitted to the appropriate review division at FDA.[10] For the three study designs, a pilot study may be useful to determine the optimal dosing duration and doses to be used in the BE study.

1. Traditional Treatment Study

The recommended design for this study is a randomized, double-blind, placebo-controlled, parallel group study with a single-blind placebo lead-in period (generally 1 to 14 days) in which efficacy and safety of the test product are assessed for a 2-week duration. Symptom assessment should be made at least twice daily (i.e., *reflective* scores) and also at the end-of-dosing interval (i.e., *instantaneous* scores). Evaluation of both reflective and instantaneous assessments of the total nasal symptom score are critical in establishing BE with a clinical endpoint. Safety measures should include physical examination, laboratory monitoring (chemistry, liver function tests, hematology, urinalysis, serum pregnancy testing in females), monitoring of vital signs, adverse event reporting, and performance of 12 lead ECGs before and after treatment with study drug.

2. Day(s) in the Park Study

The recommended design for this study is a randomized, double-blind, placebo-controlled, parallel group study in a park setting in which subjects are exposed to relevant outdoor allergens. On the study day, patients should undergo a baseline period of evaluation in the park setting to establish a minimum level of allergic rhinitis symptoms prior to randomization to study drug treatment. Patients should remain outdoors in the park for a prespecified length of time over one to two consecutive days. Nasal symptoms should be evaluated on a periodic basis throughout the full dosing interval to characterize onset of action and end-of-dosing interval efficacy. Safety assessment generally involves adverse event reporting.

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[10] A draft guidance on clinical development programs for allergic rhinitis drug therapy is under development.

3. Environmental Exposure Unit (EEU) Study

The recommended design for this study is a randomized, double-blind, placebo-controlled, parallel group study in a controlled indoor environment termed an *EEU chamber*. Repeated pretreatment exposure to the relevant allergen allows screening for symptomatic responders for enrollment in the treatment phase. On the study day, patients should be exposed to the allergen in the EEU and monitored for a baseline period to ensure a minimum level of allergic rhinitis symptoms prior to randomization to study drug treatment. Patients should remain in the EEU for a prespecified length of time over one or two days. Nasal symptoms should be evaluated on a periodic basis throughout the full dosing interval to characterize onset of action and end-of-dosing interval efficacy. Safety assessment generally involves adverse event reporting.

Subjects employed in each of the three study designs should be patients with a history of SAR, and a positive allergy test for specific allergens (e.g., allergen skin test). Patients with other significant diseases should be excluded from the study. Patients should be experiencing a defined minimum level of symptom severity at the time of study enrollment.

VII. BIOAVAILABILITY AND BIOEQUIVALENCE: PK SYSTEMIC EXPOSURE STUDIES

Plasma concentration-time profiles from BA and BE studies should be used to evaluate systemic exposure for suspension drug products that produce sufficiently high drug concentrations of the active ingredient and/or active moiety after nasal administration to obtain meaningful AUC and C_{max} data. The product quality BA study to characterize systemic exposure may be one of the same PK studies conducted to address clinical pharmacology and biopharmaceutics questions of regulatory interest. The BA study may be conducted in healthy subjects or SAR patients. The BA batch used for the PK systemic exposure study preferably should be a pivotal clinical trial batch. Alternatively, a PK batch similar to a batch used in a pivotal clinical trial may be used, in which case, any differences between the PK batch and the pivotal clinical trial batch should be discussed with appropriate CDER review staff prior to the study. If the PK batch is not one of the three batches used for the in vitro BA studies (section V.A.1), in vitro BA data should be provided for the PK batch using the same protocols as for the three batches.

For an NDA or an ANDA, the in vivo BE study should be conducted with a replicate crossover or nonreplicate crossover design. The study may be single or multiple dose. The batches of test and reference product should be batches employed in the in vitro testing. For an ANDA, the batches of test and reference used for the systemic exposure study should be the same batches used for the clinical study for local delivery, and each of these batches should be one of the three batches used for the in vitro BE studies. Subjects for the study should be healthy (non-SAR

patients), with exclusions primarily for reasons of safety. Several actuations from the drug product in each nostril may be needed to achieve measurable concentrations of the active ingredient and/or active moiety in an accessible biological fluid such as blood or plasma. For an ANDA, an IND in accordance with 21 CFR 320.31 will be needed when the number of doses in a single-dose or multiple-dose study exceed the single or total daily dose specified in the labeling of the approved NDA.

Attempts should be made in the conduct of a PK systemic exposure study to minimize loss of drug due to excess fluid drainage into the nasopharynx or externally from the nasal cavity. The bioanalytical method should be validated for accuracy, precision, specificity and sensitivity. Statistical analysis should be conducted on the log-transformed data. Average BE may be used for studies with replicate crossover or nonreplicate crossover designs. Individual BE with scaling may be used for studies with replicate crossover designs. A pilot study is recommended to assess the analytical methodology and to estimate the numbers of actuations and subjects to be used in the full-scale study.

VIII. BIOAVAILABILITY AND BIOEQUIVALENCE: PHARMACODYNAMIC OR CLINICAL STUDIES FOR SYSTEMIC ABSORPTION

A. GENERAL INFORMATION

Clinical studies for BA, or BE studies with a pharmacodynamic or clinical endpoint, are needed to assess the systemic absorption of those suspension drug products for which PK systemic exposure studies (Section VII) are not feasible. Published data suggest that systemic BE of suspension formulation antihistamine nasal products may be established based on PK data (Heykants et al., 1995). At the present time, approved nasal mast-cell stabilizer nasal spray and anticholinergic nasal spray products are solutions for which BE may be established based upon in vitro studies only. These types of studies will thus generally be needed only for corticosteroid nasal aerosols and nasal sprays. The product quality BA study to characterize systemic absorption may be one of the same clinical studies conducted to establish the safety of the active ingredient and/or active moiety in the drug product. Because this section does not provide specific recommendations for clinical studies for systemic absorption, sponsors should submit a protocol for a BE study with a pharmacodynamic or clinical endpoint for a specific drug product to the appropriate review division at FDA.

B. BE STUDY ENDPOINTS FOR CORTICOSTEROIDS

The recommended systemic absorption BE study design for nasal corticosteroids is suppression of the HPA axis. The endpoint may be either 24-hour urinary free cortisol adjusted for urinary creatinine, based on a full 24-hour urine collection, or serum cortisol levels collected every 4

hours over a 24-hour period, with exclusion of the middle of the night sample. Endpoints for placebo and test and reference treatments should be baseline-adjusted prior to statistical analyses.

C. CLINICAL STUDY BATCHES

The product quality BA batch used for the study should be a pivotal clinical trial batch used in the in vitro BA studies (section V.A). For BE studies for an NDA, the batches of T and R should be batches used in in vitro testing. For an ANDA, the batches of test and reference product used for the systemic absorption study should be the same batches used for the clinical study for local delivery. Each of these batches should be one of the three batches used for the in vitro BE studies.

D. CLINICAL STUDY DESIGNS AND SUBJECT INCLUSION CRITERIA

The study can be conducted as a placebo-controlled, randomized, multiple-dose parallel design comparing test and reference products. The study should be conducted in healthy, nonallergic volunteers not previously exposed to corticosteroids, and subjects should be domiciled within the clinical study center during the dosing days. Three treatments, test and reference products at the labeled dose (maximum labeled dose when labeling includes more than one dose) and a placebo of the test product, should be used. Each treatment period should consist of 14 days of dosing. Timed urine or serum samples for determination of 24-hour urinary free cortisol or 24-hour serum cortisol levels should be collected prior to dosing (baseline) and during the last 24-hours of the 14 days of dosing. In addition, we recommend determining two to three interval 24-hour urinary free cortisol or 24-hour serum cortisol levels (e.g., performing additional assessments on days 4, 7, and/or 10) to better profile the onset of the effect of test and reference products, should detectable adrenal suppression occur.

Alternatively, the study could be conducted as a placebo-controlled, randomized, multiple-dose crossover design comparing test and reference (Wilson et al., 1998). As in the parallel design study, the study should be conducted in healthy, non-allergic volunteers not previously exposed to cortocosteroids. During the dosing days, subjects should be domiciled within the clinical study center to ensure compliance with the study protocol. Three treatments, test and reference at the labeled dose (maximum labeled dose when labeling includes more than one dose), and a placebo of the test product should be used. Each treatment period should consist of 14 days of dosing. A shorter dosing duration would be considered with adequate scientific justification. Washout periods between treatments should be adequate to eliminate the possibility of a carryover effect. Urine or serum samples for determination of 24-hour urinary free cortisol or 24-hour serum cortisol levels should be collected prior to each dosing period (baseline data) and during the last 24-hours of each dosing period. In addition, we recommend determining two to three interval 24-hour urinary free cortisol or 24-hour serum cortisol levels (e.g., performing additional assessments on days 4, 7, and/or 10) to better profile the onset of the effect of test and reference products,

should detectable adrenal suppression occur.

IX. STATISTICAL ANALYSES

In vitro studies yield both profile and nonprofile data, which require different statistical analyses. Noncomparative BA in vitro data analyses for both profile and nonprofile data are discussed in section IX.A. For BE studies, methods of comparison for nonprofile analyses are discussed in section IX.B, for supportive nonprofile and profile analyses in section IX.C, and for profile analyses in section IX.D. Methods for comparison of categorical endpoints from the SAR studies are discussed in section IX.E.

A. IN VITRO BA DATA

Means, SDs, and percent CVs should be reported for the measures recommended in this guidance to document BA.

μ_T	=	T means (log scale)
σ_{BT}	=	T between batch standard deviations (log scale)
σ_{CT}	=	T between canister standard deviations (log scale)
σ_{LT}	=	T within canister between life stage standard deviation

The overall means for the formulation should be averaged over all bottles or canisters, life stages (except for priming and repriming evaluations), and batches. In addition to overall means, means at each lifestage for each batch averaged over all bottles or canisters, and for each lifestage averaged over all batches, are requested. For profile data, means, SDs, and percent CVs should be reported for deposition in each of Groups 1, 2 and 3 of the CI or MSLI data, as well as on the individual accessories and stage within Group 1.

B. IN VITRO BE DATA: NONPROFILE ANALYSES USING A CONFIDENCE INTERVAL APPROACH

Nonprofile analyses should be applied to the following tests: (1) dose or spray content uniformity through container life; (2) droplet size distribution; (3) spray pattern; and (4) priming and/or repriming, when this information is specified in the labeling.

1. Study Protocol

Data for the BE criterion should be based on testing a suitable number of bottles or canisters from each of three batches of the T and R drug products. Each bottle or canister should be tested for the measure (parameter) of interest at beginning and end, or

beginning, middle, and end of unit life, as indicated in section V and Table 1. Rather than evaluate performance at each life stage separately, a criterion is recommended that combines the multiple life stages. In doing so, the multiple life stages are considered as providing measures of the same underlying quantity. The recommended criterion considers deviations from uniformity across bottle or canister life stages; results are ideally uniform. Lack of uniformity between life stages should be treated as another variance component in the criterion.

For suspension formulation nasal sprays and solution formulation and suspension formulation nasal aerosols, the number of canisters or bottles (units) of product to be studied should not be fewer than 30 for each of the test and reference products (i.e., no fewer than 10 from each of three batches). For solution formulation nasal sprays, no fewer than 10 units from each of the three batches or three sublots should be studied. The number of units is a function of T to R product means and variances. Estimates of these mean differences and variances will necessitate pilot studies.

2. Criterion for Comparisons, Confidence Interval, and Bioequivalence Limit

The equivalence approach for nonprofile tests relies on (1) a criterion to allow the comparison, (2) a confidence interval for the criterion, and (3) a BE limit for the criterion.

a. Criterion for comparison

The in vitro population BE criterion and BE limit are:

$$\frac{(\mu_T - \mu_R)^2 + (\sigma_T^2 - \sigma_R^2)}{\sigma_R^2} \leq \theta$$

where:

μ_T, μ_R	=	T and R means (log scale)
σ_{BT}, σ_{BR}	=	between batch T and R standard deviations (log scale)
σ_{CT}, σ_{CR}	=	between canister T and R standard deviations (log scale)
σ^2_R	=	$\sigma^2_{BR} + \sigma^2_{CR} + \sigma^2_{LR}$
σ^2_T	=	$\sigma^2_{BT} + \sigma^2_{CT} + \sigma^2_{LT}$
σ_{LT}, σ_{LR}	=	within T and R canister between life stage standard deviation
θ	=	in vitro BE (upper) limit

The overall means for the two formulations should be averaged over all bottles or canisters, life stages (except for priming and repriming evaluations), and batches.

The general approach should be to calculate a 95 percent upper bound for the criterion. If this upper bound is less than or equal to the upper limit, θ, the test product may be judged to be bioequivalent to the reference product at the 5 percent level. The criterion will be further discussed in the guidance for industry on *In Vivo Bioequivalence Studies Based on Population and Individual Bioequivalence Approaches* (draft December 1997), when finalized. A population, rather than average, bioequivalence criterion is recommended in order to estimate whether the test product may be more variable than the reference product. The test product should be as or more consistent in the delivery of drug than is the reference product. An individual BE approach is not appropriate for in vitro data because there are no subjects, thus no subject-by-formulation interaction.

b. Determining a 95 percent upper bound

CDER recommends that a method of moments approach be used for estimating the means and variances needed to determine the population bioequivalence criterion. Approaches based on restricted maximum likelihood (REML) may be used in special cases. For determining the 95 percent upper bound, CDER recommends using a method analogous to one proposed for individual bioequivalence (Hyslop, Hsuan and Holder 1998).

c. Specification of the population BE upper limit

The general form of the upper limit, θ, is analogous to the form of the population BE criterion, which is

$$\frac{(\text{mean difference in natural log scale})^2 + \text{variance terms}}{\text{comparison variance}}$$

The corresponding form for the upper limit is then

$$\frac{(\text{average BE limit in natural log scale})^2 + \text{variance terms offset}}{\text{scaling variance}}$$

This formula contains three values to be specified: (1) average BE limit, (2) variance terms offset, (3) and scaling variance. These values will be specified when this guidance is finalized based on simulation work now in progress.

Average BE Limit

Due to the low variability of in vitro measurements, at the present time CDER recommends that the limit not be be larger than 90/111 (i.e., the ratio of geometric means would fall within 0.90 and 1.11). A value of 0.90 is tentatively recommended as the average BE limit. This value should be used in calculating the population BE limit (refer to θ in the equation in section IX.B.2.a, above).

Variance Terms Offset

This value arises to allow some difference among the total variances that may be inconsequential. In this regard, the variance terms offset is analogous to the average BE limit. The variance terms offset also helps correct for the effect on power and sample size for the need to estimate the variances. Because of the low variability of in vitro measurements, the variance terms offset, denoted ϵ_P in the draft guidance on *In Vivo Bioequivalence Studies Based on Population and Individual Bioequivalence Approaches* (December 1997), when finalized, should be taken as 0.0. CDER is also considering ϵ_P equal to 0.01.

Scaling Variance

This value adjusts the BE criterion depending on the reference product variance. When this variance is greater than the scaling variance, σ_{T0}^2, the limit is widened. When this variance is less than the scaling variance, the limit is narrowed.

Mixed scaling should be employed for in vitro studies, as described in the draft guidance on *In Vivo Bioequivalence Studies Based on Population and Individual Bioequivalence Approaches* (December 1997), when finalized. With mixed scaling, when the reference variance ***in the study*** is less than the scaling variance, the population BE criterion should be modified to its *constant-scaled* form:

$$\frac{(\mu_T - \mu_R)^2 + (\sigma_T^2 - \sigma_R^2)}{\sigma_{T0}^2}$$

Mixed scaling is used to avoid penalizing test products for cases with very low reference variance. It is CDER's current intent to select σ_{T0} for in vitro studies so that most studies will use constant scaling and thus, that σ_{T0} will be at least 0.10.

The upper limit may be interpreted by reference to a population distance ratio (PDR). The PDR is the ratio of the test-reference distance (in the log scale) to the reference-reference distance. In contrast to individual BE, the distances for population BE are based on administration to separate individuals (further details will be provided in the guidance for industry on *In Vivo Bioequivalence Studies Based on Population and Individual Bioequivalence Approaches* (draft December 1997), when finalized. The population BE criterion, denoted by PBC, is related to the PDR by

$$PDR = (1 + \frac{PBC}{2})^{\frac{1}{2}}$$

Substituting the BE limit θ for PBC expresses the upper limit in the PDR scale. The specification of 0.90 for the average limit, 0.0 for the variance offset, and 0.10 for the scaling standard deviation corresponds to an upper limit for PDR of 1.25.

C. IN VITRO BE DATA: SUPPORTIVE NONPROFILE AND PROFILE ANALYSES

The following tests provide supportive characterization data: (1) plume geometry; (2) tail off profile; (3) priming data, when reference product labeling does not specify priming information; and (4) drug CMD and drug and aggregate PSD data from microscopic analyses. The comparative data requested in section V should be provided, based upon the same number of bottles or canisters recommended in the protocol of section IX.A.1. Statistical criteria need not be applied.

D. IN VITRO BE DATA: PROFILE ANALYSES USING A CONFIDENCE INTERVAL APPROACH

Profile analyses apply to cascade impactor (CI) or multistage liquid impinger (MSLI) data for nasal aerosols and nasal sprays. Analyses may rely on a criterion for comparisons of means and variances relative to a BE limit, with calculation of a 90 percent confidence interval. The general approach is adaptable to cascade impactors of varying numbers of stages and accessories, or groups of stages and accessories. As discussed in section V.B.2, profile comparisons may be based on drug deposition within three groups.

1. Study Protocol

Data for the BE criterion should be based on testing a suitable number of bottles or

canisters from each of three batches of the T and R drug products (or three sublots for solution formulation nasal sprays). Each canister should be tested for deposition at the beginning and end life stages, as indicated in section V.B.2. The number of canisters to be studied from each batch, which should not be less than 10, is a function of test to reference product means and variances. Estimates of these mean differences and variances will require pilot studies.

2. Criterion for Comparison

The criterion considered appropriate for the profile comparison is:

$$Rd \leq \theta$$

where:

Rd	=	in vitro BE criterion
θ	=	in vitro BE limit

Rd is derived with the following notation:

Let:

P_R, P_T	=	population mean profile across the batches of the reference product and test product
F_{BR}, F_{BT}	=	distribution of deviation of batch mean from population mean profile of the reference product and test product
F_{CR}, F_{CT}	=	distribution of deviation of canister mean from batch mean profile of reference product and test product
p_R, p_T	=	observed profile of a given puff of reference product and test product (i.e., p_R has a compound distribution of MN(100, P_R), F_{BR} and F_{CR}, and p_T has a compound distribution of MN(100, P_T), F_{BT} , and F_{CT}, where MN(100, P) is a multinomial distribution with n=100 and P=(p_1, p_2, …, p_S) for an impactor of S stages)

and:

d_{TR}	=	observed distance between p_R and p_T, the test-reference distance
$d_{RR'}$	=	observed distance between p_R and $p_{R'}$, the reference-

reference distance (i.e., reference-reference deviation)

rd = $d_{TR}/d_{RR'}$, observed ratio of test-reference distance to reference-reference deviation

The in vitro BE measure is defined by

Rd = E(rd)

where:

E(rd) = expected value of rd

Further information on d_{TR}, $d_{RR'}$, rd, and the in vitro profile comparison procedure is provided in Appendix A.

3. Determining a 95 Percent Upper Bound

Since there is no exact or asymptotic distribution of the average rd, the 95% upper bound should be determined by the 95th percentile of the empirical sampling distribution generated by a random sample of the matched triplet (test, reference 1, reference 2) of canisters. A description of the procedure is provided in Appendix B.

4. Specification of the Upper Limit

Reserved (simulation studies to develop specifications for the upper limit are ongoing).

E. IN VIVO BE DATA: CATEGORICAL ENDPOINTS

Reserved (statistical analyses are under development).

X. MULTIPLE STRENGTHS

A small number of nasal sprays for local action are available in two strengths. Current examples are: (1) ipratropium bromide nasal spray, a solution formulation; and (2) beclomethasone dipropionate nasal spray, a suspension formulation. Lower strengths of a product ordinarily would achieve the lower dose per actuation using a lower concentration formulation, without changing the actuator and metering valve or pump (other than diptube) used in the higher strength product. The following sections describe recommended BA and BE studies for low strengths of nasal sprays for which BA or BE for the higher strengths has previously been established.

Recommendations are also provided for cases in which BA or BE is initially established on the low-strength product. No approved nasal aerosols are available in multiple strengths, thus BA and BE recommendations are not considered for these products.

A. SOLUTION FORMULATION NASAL SPRAYS

BA of lower or higher strength solution formulation nasal sprays should be based on conduct of all applicable in vitro tests described in section V. These studies are generally noncomparative in character. Documentation of BE between T and R products should follow the recommendations described in section III regarding formulation and container and closure system. Abbreviated in vitro testing (section V) is recommended to document BE of the low-strength T product to the low-strength R product, provided BE of the high-strength product has been documented.

In vitro test	High Strength	Low Strength
Dose content uniformity	BE[1]	BE
Priming and repriming	Yes	Yes
Tail off	Yes	Yes
Droplet size distribution		
By laser diffraction	BME	B
By cascade impactor	BE	No
Spray pattern	BE	B
Plume geometry	B	No

[1] Beginning (B), Middle (M), End (E)

With the exception of the reduced testing, the same protocols and acceptance criteria used to establish BE of the high strength products should be used for the low strength products. In vivo studies are not needed for documentation of BA or BE of solution formulation nasal sprays. For cases in which BE is documented for the low-strength product, to subsequently document BE for the high-strength product, all applicable in vitro tests described in section V should be conducted.

B. SUSPENSION FORMULATION NASAL SPRAYS

BA of lower strength suspension formulation nasal sprays should be based on conduct of all applicable in vitro tests described in section V and systemic exposure studies, assuming availability of bioanalytical methodology to allow measurement of systemic concentrations. In the absence of this methodology, BA for systemic absorption should be documented through clinical studies. BE conditions for the lower strength product should be the following:

1. Documentation of BE for the high-strength test products and high-strength reference

products, based on acceptable comparative formulations and container and closure systems, comparative in vitro data, and comparative in vivo data

2. Acceptable comparative formulations and container and closure systems for the low-strength test products and low-strength reference products

3. Acceptable comparative studies for low-strength test products and low-strength reference products for all applicable in vitro tests in section V

4. Proportionally similar unit dose between high- and low-dose test product and high- and low-dose reference product

5. Equivalent droplet and drug PSD between high- and low-dose test product and high- and low-dose reference product

Provided the above conditions are met, in vivo studies are not needed for documentation of BE of the lower strength products.

For cases in which an ANDA applicant initially documents BE on the low-strength product, and subsequently submits an ANDA for the high-strength product, full in vitro and in vivo documentation of BE should be provided for the high-strength product. For cases in which an ANDA applicant has documented BE for its high-strength product and wishes to conduct applicable in vitro tests and in vivo study on the low-strength product, BE criteria need not include in vitro comparisons between high- and low-strength products.

XI. SMALLER CONTAINER SIZES

Nasal aerosols and nasal sprays may be available in two container sizes. Current examples are: (1) beclomethasone dipropionate nasal aerosol, a suspension formulation; (2) fluticasone propionate nasal spray, a suspension formulation; and (3) cromolyn sodium nasal spray, a solution formulation. Smaller container sizes of nasal aerosols should be formulated with the same components and composition, metering valve, and actuator as the large container size that was studied in pivotal clinical trials (NDA) or for which BE has been documented (ANDA). Smaller container sizes of nasal sprays should be formulated with the same components and composition, pump, and actuator as the large container size that was studied in pivotal clinical trials (NDA) or for which BE has been documented (ANDA). Where this is the case, no further documentation of either BA or BE is necessary. However, reestablishing proper priming, given a change in the dead volume of the pump and actuator, may in some cases be appropriate (see section V.B.5).

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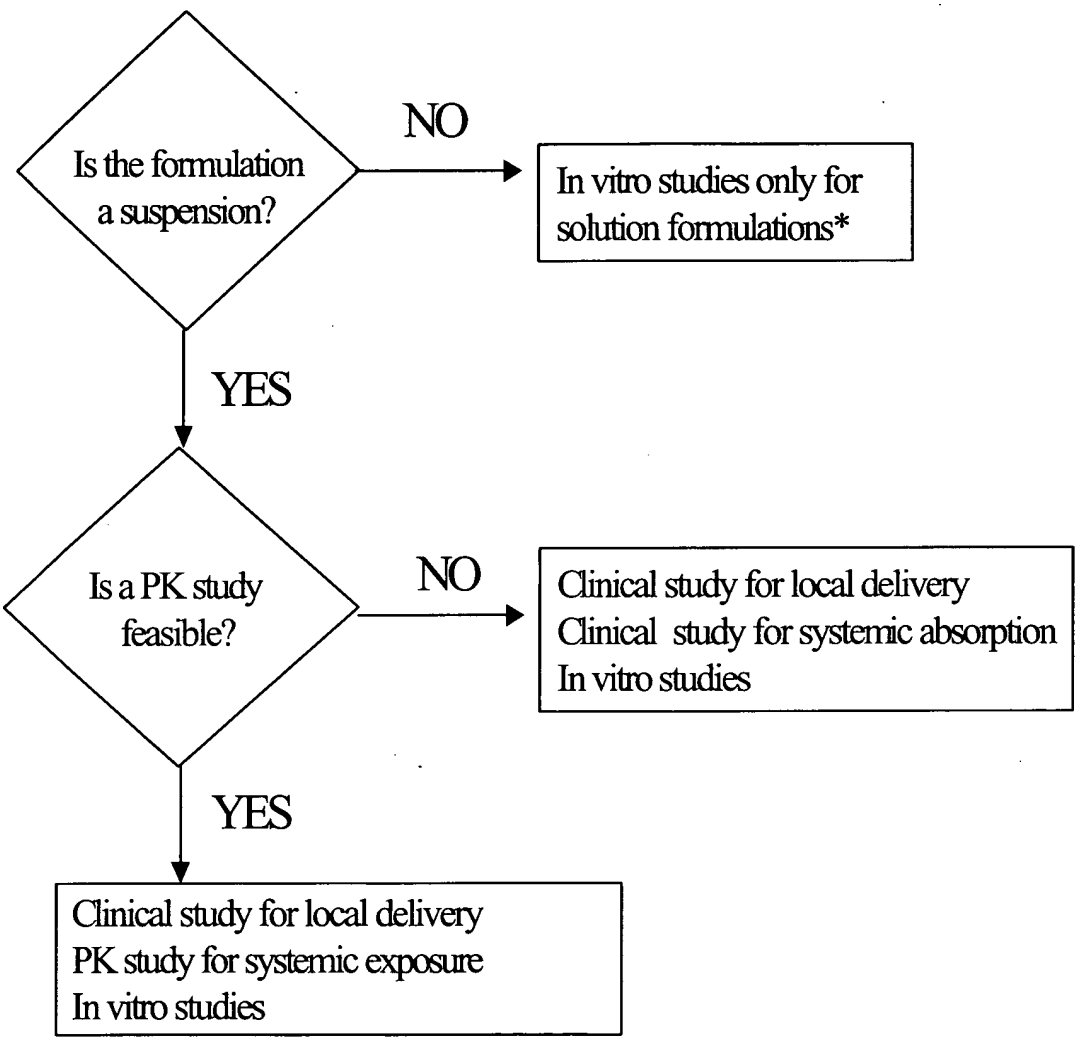
Decision Tree For Product Quality
BA and BE Studies For Nasal Aerosols and Nasal Sprays



*See Section II (A) regarding additional in vivo BA studies needed for solution and suspension formulations.

Table 1
In Vitro BA and BE Studies for Nasal Aerosols and Nasal Sprays

TEST	BA AND BE STUDY MEASURE(S)[1]	BE MEASURES[2]	LIFESTAGE(S) B (beginning) M (middle) E (end)	CONFIDENCE INTERVAL OR SUPPORTIVE CHARACTERIZATION FOR BE	GUIDANCE SECTIONS
Dose or spray content uniformity through container life	Drug mass per single dose	Same as previous column	B, M, E (aerosols) B, E (sprays)	Confidence interval	V.B.1, IX.B
Droplet size distribution	D_{10}, D_{50}, D_{90}, span	D_{50}, span	B, M, E	Confidence intervals	V.B.2, IX.B
Particle size distribution (CI or MSLI)	Deposition profile over 3 groups	Same as previous column	B, E	Confidence interval	V.B.2, IX.D
Drug and aggregate PSD of suspensions (light microscopy)	Drug CMD and GSD; aggregate PSD	Drug CMD	B	Supportive characterization	V.B.2, IX.C
Spray pattern	D_{max}, D_{min}, ovality ratio	D_{max} or D_{min}, ovality ratio	B, E	Confidence intervals	V.B.3, IX.B
Plume geometry	Length, width, spray cone angle (if feasible)	Same as previous column	B	Supportive characterization	VB.4, IX.C
Priming and repriming	Drug mass per single actuation	Same as previous column for: • priming, and • repriming if in precursor product (R) labeling	From first actuation (priming); from first actuation after specified period of nonuse (repriming)	Confidence interval for priming and repriming if in precursor product (R) labeling; supportive characterization of priming when not in labeling	V.B.5, IX.B, IX.C
Tail off	Drug mass per single actuation	Same as previous column	From end of labeled number of actuations to exhaustion	Supportive characterization	V.B.6, IX.C

[1] Data requested as part of the BA or BE submission.
[2] Measures requested for comparative in vitro BE documentation.

APPENDIX A
IN VITRO PROFILE COMPARISON PROCEDURE BASED ON CHI-SQUARE DIFFERENCES

This appendix describes a method of comparing cascade impactor (CI) or multistage liquid impinger (MSLI) deposition profiles on "S" stages or accessories, or groups of stages and accessories, from droplet and/or particle sizing studies. Equivalence may be assessed by comparing the profile difference between test product and reference product canisters (nasal aerosols) or bottles (nasal sprays) to the profile variation between reference product canisters or bottles. The profile comparison is based on chi-square differences.

The following table represents the population mean profiles P_T and P_R of one test canister and one reference canister, respectively.

Product	Stage									
	1	2	3	4	. . .	s	. .	S	Total
Test	P_{T1}	P_{T2}	P_{T3}	P_{T4}	. .	P_{Ts}	. .	P_{TS}	100
Reference	P_{R1}	P_{R2}	P_{R3}	P_{R4}	. .	P_{Rs}	. .	P_{RS}	100

The profile difference between test and reference product canisters is assessed by the chi-square measure as follows:

$$D_{TR} = (P_{T1} - P_{R1})^2/((P_{T1} + P_{R1})/2) + (P_{T2} - P_{R2})^2/((P_{T2} + P_{R2})/2) + \ \ldots \ +$$

$$(P_{TS} - P_{RS})^2/((P_{TS} + P_{RS})/2)$$

Similarly, the profile variation (i.e., difference) between any two canisters of the reference product is:

$$D_{RR'} = (P_{R1} - P_{R'1})^2/((P_{R1} + P_{R'1})/2) + (P_{R2} - P_{R'2})^2/((P_{R2} + P_{R'2})/2) + \ \ldots \ +$$

$$(P_{RS} - P_{R'S})^2/((P_{RS} + P_{R'S})/2)$$

The approach involves a comparison of D_{TR}, the profile difference between one test canister and one reference canister, to $D_{RR'}$, the profile variation between two canisters of the reference product, where the latter is based on two randomly selected reference canisters. The comparison of profile differences is given by the ratio of D_{TR} to $D_{RR'}$. A large D_{TR} is one that is large relative to the variation that would be expected between two canisters of the reference product.

In order to estimate D_{TR} and $D_{RR'}$, the observed data of one canister of test product and two different canisters of reference product need to be matched as a triplet. The observed profiles of the three canisters of a given triplet may be represented in the following table.

Product	Stage										
	1	2	3	4	.	.	s	.	.	S	Total
Test	p_{T1}	p_{T2}	p_{T3}	p_{T4}	.	.	p_{Ts}	.	.	p_{TS}	100
Reference 1	p_{R1}	p_{R2}	p_{R3}	p_{R4}	.	.	p_{Rs}	.	.	p_{RS}	100
Reference 2	$p_{R'1}$	$p_{R'2}$	$p_{R'3}$	$p_{R'4}$	.	.	$p_{R's}$	.	.	$p_{R'S}$	100

The observed profile difference d_{TR} between test and reference products is:

$$d_{TR} = (p_{T1} - (p_{R1} + p_{R'1})/2)^2/((p_{T1} + (p_{R1} + p_{R'1})/2)/2) + (p_{T2} - (p_{R2} + p_{R'2})/2)^2/((p_{T2} + (p_{R2} + p_{R'2})/2)/2)$$

$$+ \ \ldots \ + (p_{TS} - (p_{RS} + p_{R'S})/2)^2/((p_{TS} + (p_{RS} + p_{R'S})/2)/2).$$

The reference product canister-to-canister variation within the triplet is estimated by the profile difference between the two paired reference canisters, R and R':

$$d_{RR'} = (p_{R1} - p_{R'1})^2/((p_{R1} + p_{R'1})/2) + (p_{R2} - p_{R'2})^2/((p_{R2} + p_{R'2})/2) + \ \ldots \ + (p_{RS} - p_{R'S})^2/((p_{RS} + p_{R'S})/2).$$

For a given triplet of canisters (Test, Reference 1, Reference 2), the ratio of d_{TR} to $d_{RR'}$ may be obtained as follows:

$$rd = d_{TR}/d_{RR'}.$$

Assuming that there are N(T, R, R') triplets in the sample, the unbiased estimate of Rd [=E(rd)] is the sample mean of the N observed $d_{TR}/d_{RR'}$ values.

For an experiment consisting of three lots each of test and reference products, and with 10 canisters per lot, the lots can be matched into six different combinations of triplets with two different reference lots in each triplet. The 10 canisters of a test lot can be paired with the 10 canisters of each of the two reference lots in (10 factorial)^2 = $(3{,}628{,}800)^2$ combinations in each of the lot-triplets. Hence a random sample of the N canister-pairing of the six Test-Reference 1-Reference 2 lot-triplets is needed. Rd is estimated by the sample mean of the rd's calculated for the triplets in the selected sample of N:

$\hat{}Rd$ = sample mean of $(d_{TR}/d_{RR'})$.

APPENDIX B

DETERMINATION OF THE 95% UPPER BOUND FOR IN VITRO PROFILE COMPARISONS

Assume the profile comparison is to be carried out with a random sample with no replacement of N = 500 matches (from the population of 6 x (10 factorial)^2 matches). The average of the 500 sample rd's (= d_{TR} /d_{RR}.) gives ^Rd. The 95% upper bound of Rd is the 95th percentile of the 500 calculated rd's (i.e., the 25th largest rd among the 500 calculated rd's).